UNIVERSIDAD COMPLUTENSE DE MADRID
FACULTAD DE ODONTOLOGÍA

TESIS DOCTORAL

Cambios volumétricos y perfilométricos en estudios experimentales in vivo de regeneración ósea y de peri-implantitis

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

Riccardo Di Raimondo

DIRECTOR

Mariano Sanz Alonso

Madrid

UNIVERSIDAD COMPLUTENSE DE MADRID

Facultad de Odontología

Departamento de Estomatología III

CAMBIOS VOLUMÉTRICOS Y PERFILOMÉTRICOS EN ESTUDIOS EXPERIMENTALES IN VIVO DE REGENERACIÓN ÓSEA Y DE PERI-IMPLANTITIS

Tesis Doctoral

MEMORIA PARA OPTAR AL GRADO DE DOCTOR PRESENTADA POR

Riccardo Di Raimondo

Director

Prof. Mariano Sanz Alonso

Madrid 2020

AGRADECIMIENTOS

Antes de empezar quiero decir que esta tesis es el resultado de la colaboración de muchas personas que, gracias a sus enseñanzas me han permitido llegar aquí.

En primer lugar, quiero nombrar a mi director, el Prof. Mariano Sanz Alonso. Recuerdo como si fuera ayer el día en el que el me propuso empezar un proceso de aprendizaje como este. Desde el primer momento no tuve ninguna duda a respeto y acepté comprometerme con él y con todo el equipo de investigación para poder aprender de los mejores.
El Prof. Mariano Sanz Alonso ha sido un pilar para mi aprendizaje, me hizo crecer no solo como investigador, sino también humanamente, tratándome como un hijo y mejorándome desde todos los puntos de vista y contribuyendo a la persona que soy hoy, gracias a todos sus consejos, apoyo y paciencia a lo largo de estos años.

Quiero nombrar a Javier Sanz Esporrín que, aunque no somos hermanos "de sangre", en mi corazón lo somos igualmente. El me ha enseñado muchísimo desde las bases de la metodología, y siguiendo hasta como mejorar la forma de escribir un artículo, además de ayudarme en todos los estudios de mi tesis. Tener la oportunidad de aprender de él es un privilegio infinito. También tengo que agradecerle por la paciencia que ha tenido siempre conmigo y contribuyendo a mi maduración no sólo como investigador sino también como persona.

También quiero agradecer al Dr. Ignacio Sanz Martin, por todas sus enseñanzas, pero sobre todo por el apoyo y paciencia incondicionales prestadas a lo largo de este tiempo, gracias a la cual he podido aprender toda la metodología aplicada en todos los artículos de mi tesis. Sin él, seguramente no hubiera llegado a este día y al bagaje que tengo ahora.

Quiero nombrar al Prof. Fernando Muñoz que ha sido siempre muy amable conmigo, enseñándome a usar los programas que dispone en la Universidad de Lugo y dándome también la posibilidad de usarlos para las mediciones de mis estudios. Pero lo que más quiero agradecerle, es que cuando fui a Lugo me hizo sentir en mi casa en todos los días de mi permanencia ahí.

También quiero nombrar al Dr. Fabio Vignoletti que no solo me ha enseñado como interpretar las histologías, sino también por apoyarme durante estos años y compartiendo sus conocimientos también conmigo, y mejorándome como investigador.

Quiero agradecer a todos los miembros del grupo de Investigación de la Universidad Complutense de Madrid, sobre todo a nuestro fantástico "grupo de investigación preclínica". Fernando Luengo y Rafael Plá que, a parte de ser grandes amigos, hemos podido crecer juntos en este grupo como investigadores. Pero especial importancia, al Dr. Javier Nuñez que ha sido siempre presente en todas nuestras investigaciones, y no solo nos ha enseñado muchísimo de lo que él sabe, sino también ha tenido una paciencia incondicional con todos nosotros, a veces actuando como un "padre" para controlar y gestionarnos cuando nos portábamos de manera incorrecta. Este grupo de Investigación, compuesto por supuesto también por el Prof. Mariano Sanz Alonso, el Dr. Fabio Vignoletti y Javier Sanz Esporrin ha sido y será siempre para mi una segunda familia y con ellos hemos vivido experiencias inolvidables, no solo formativas sino sobre todo humanas, empezando en el Hospital Gomez Ulla, y siguiendo en Cáceres y a Lugo.

Quiero agradecer a mi familia, en especial a mi padre y mi madre por haberme apoyado en todos estos años lejos de casa, por haberme motivado no solo en los momentos más fáciles, sino también en aquellos momentos difíciles. Ellos han sido y siguen siendo mis pilares y estoy muy orgulloso de los que me han enseñado en estos treinta años, y muy contento de llegar aquí con

ellos a mi lado. También quiero agradecer a mi hermano y mi abuela que siempre me han apoyados y me dijeron de seguir mis objetivos y luchar por ellos.

Por último, quiero nombrar a todos mis amigos y amigas que han creído en mi y han tenido en los últimos años mucha paciencia conmigo en “aguantarme” en mis momentos más estresantes.

A todos, de verdad Gracias.

PREFACIO

La presente tesis doctoral está basada en tres publicaciones científicas:

Artículo 1

Alveolar crests contour changes after guided bone regeneration using different biomaterials: an experimental in vivo investigation. Di Raimondo R, Sanz-Esporrin J, Pla R, Sanz-Martin I, Luengo F, Vignoletti F, Nuñez J, Sanz M. Clin Oral Investig (2020) 24 (7): 2351-2361

Artículo 2

Hard and soft tissue changes after guided bone regeneration using two different barrier membranes. An experimental in vivo investigation. Di Raimondo R, Sanz-Esporrin J, Sanz-Martin I, Pla R, Luengo F, Vignoletti F, Nuñez J, Sanz M. Clin Oral Investig (2020) doi: 10.1007/s00784-020-03537-5

Artículo 3

Hard tissue volumetric and soft tissue contour linear changes at implants with different surface characteristics after experimentally peri-implantitis. An erimental in vivo investigation. Di Raimondo R, Sanz-Esporrin J, Sanz-Martin I, Vignoletti F, Nuñez J, Muñoz F, Haugen HJ, Sanz M. Clin Oral Investig (Accepted with minor changes)

ÍNDICE DE CONTENIDOS

I. RESUMEN

Antecedentes. Tras la pérdida dentaria se producen alteraciones dimensionales de las crestas óseas residuales, con pérdida de la disponibilidad ósea para colocar implantes dentales, lo que requiere procedimientos quirúrgicos regenerativos. Igualmente, las lesiones óseas resultantes de la peri-implantitis pueden requerir intervenciones regenerativas. Aunque el análisis histológico en estudios experimentales in vivo es el "*gold standard*" para evaluar la cicatrización de los tejidos, los avances en la tecnología digital nos permiten en la actualidad obtener información de los cambios de volumen y contorno, de ambos tejidos blandos y duros durante los procesos de cicatrización, de una modo menos invasivo y más preciso.

Objetivos. Evaluar el comportamiento de los tejidos blandos tras técnicas de regeneración ósea horizontal, mediante dos diferentes combinaciones de biomateriales (estudio 1); evaluar el comportamiento de ambos tejidos blandos y duros tras técnicas de regeneración ósea horizontal, mediante dos membranas barrera diferentes (estudio 2); evaluar el comportamiento de ambos tejidos blandos y duros en un modelo de peri-implantitis experimental, con dos superficies diferentes de implantes (estudio 3).

Material y Métodos. En las tres investigaciones se utilizó un modelo experimental in vivo (perros "Beagle"). El análisis del cambio del contorno de los tejidos blandos se realizó a diferentes alturas en los modelos digitalizados (STL) superpuestos, a partir de impresiones de silicona obtenidas en cada visita de los estudios y se evaluaron los cambios que ocurrieron en distintos momentos de la curación, de acuerdo con las intervenciones quirúrgicas realizadas. Los análisis volumétricos de los tejidos duros se realizaron mediante el uso del Micro-ct, a partir de los bloques de tejidos obtenidos al final de cada investigación.

Resultados. *Estudio 1:* los cambios del contorno de los tejidos blandos fueron superiores en los grupos donde se usó la combinación de un sustituto óseo y una membrana barrera (test y control positivo), en comparación con el control negativo, aunque las diferencias fueron significativas solamente después del periodo de curación largo. *Estudio 2:* los cambios

volumétricos y del contorno de los tejidos blandos y duros fueron superiores en los grupos donde se usó la combinación de un sustituto óseo y una membrana barrera (test y control positivo), en comparación con la membrana sola (control negativo), aunque las diferencias entre los grupos no fueron significativas. *Estudio 3:* los cambios volumétricos y del contorno de los tejidos blandos y duros fueron similares entre las dos superficies de implantes, sin diferencias significativas.

Conclusiones. La regeneración ósea guiada con un sustituto óseo asociado a una membrana barrera, se considera el tratamiento de elección, ya que los cambios volumétricos y del contorno de ambos tejidos blandos y duros de los grupos test y control positivo de ambos estudios fueron superiores (estudios 1 y 2). También, se puede concluir que la superficie tratada con una capa mono molecular de multi-fosfonatos no aporta beneficios, en términos de cambios del contorno de los tejidos blandos y pérdida de hueso tridimensional, ya que los resultados entre los grupos fueron similares (estudio 3). Por último, se confirma la validez de la metodología utilizada en estudios experimentales in vivo para evaluar el comportamiento de ambos tejidos blandos y duros.

PALABRAS CLAVES: Implantes dentales, superficies implantarias, superficies bioactivas, regeneración ósea guiada, ROG, defectos óseos, cambios volumétricos, análisis perfilométricos, injertos óseos, membranas barrera, tecnología digital, escáner, peri-implantitis, estudios experimentales.

II. ABSTRACT

Background. Tooth loss causes dimensional alterations of the alveolar process, which result in hard tissue deficiencies that could difficult the placement of dental implants. In these situations, lateral bone augmentation is used to achieve the appropriate alveolar bone crest. Similarly, bone defects resulting from peri-implantitis may require regenerative interventions. Histological analysis in experimental studies is considered the gold standard to assess tissues healing, however the improvement in digital technology could allow us to obtain more information about volumetric and contour changes of both soft and hard tissues during healing processes, in a less invasive and more precise way.

Objective. To evaluate the contour linear changes of soft tissues after lateral bone regeneration, using two different combinations of biomaterials (study 1); evaluate the volumetric and contour linear changes of both soft and hard tissues after lateral bone regeneration, using two different barrier membranes (study 2); evaluate the volumetric and contour linear changes of both soft and hard tissues in an experimental peri-implantitis model, with two different implant surfaces (study 3).

Material and methods. Eight "Beagle" dogs were used in each investigation. To perform soft tissue analysis silicon impressions of the mandibles were taken at each study visit and poured with stone. Then, different measurements were obtained on the superimposed digitalized cast models (STL), according to the type of the investigation (guided bone regeneration or experimental peri-implantitis). The volumetric analysis of hard tissues was performed by means of Micro-Ct only at the end of each investigation.

Results. *Study 1:* soft tissue contour linear changes were superior in the groups where a combination of both a bone graft and a barrier membrane were used (test and positive control), compared to the negative control, although differences were statistically significant only after the long healing period. *Study 2:* volumetric and contour linear changes of both soft and hard tissues were superior in the groups where a combination of both a bone graft and a barrier

membrane were used (test and positive control), compared to the membrane alone (negative control), although differences were not statistically significant. *Study 3:* volumetric and contour linear changes of both soft and hard tissues were similar between the two implant surfaces, without statistically significant differences between them.

Conclusions. Guided bone regeneration with the combination of both a bone graft and a membrane is considered the treatment of choice, since in both studies volumetric and contour linear changes of both soft and hard tissues were greater in the test and positive control groups (studies 1 and 2). Moreover, we conclude that the phosphonate-rich surface did not provide benefits, against experimental peri-implantitis, when evaluated the soft tissue contour changes and tridimensional bone loss, since the results between the groups were similar (study 3). Finally, we confirm the validity of the methodology to assess the behaviour of both soft and hard tissues in experimental in vivo studies.

KEY WORDS: Dental implants, implant surfaces, bioactive surfaces, guided bone regeneration, GBR, bone defects, volumetric analysis, contour changes, bone grafts, barrier membranes, digital technology, scanner, peri-implantitis, experimental models.

III. INTRODUCCIÓN

Alteraciones dimensionales tras extracción o pérdida dentaria

Está ampliamente demostrado, tanto en estudios preclínicos como clínicos, que la periodontitis en sus etapas avanzadas, así como traumatismos, enfermedades congénitas, fracturas de raíz y fracasos endodónticos, pueden conducir a la pérdida de los dientes afectados, lo que deriva en alteraciones dimensionales de los tejidos blandos y duros, tanto a nivel horizontal como vertical (Hammerle & Tarnow 2018).

Estos cambios ocurren tanto en la superficie exterior, como en el interior del alveolo clasificándose así en: intra-alveolares y extra-alveolares.

a) Cambios intra-alveolares

Tras la exodoncia o pérdida dentaria, dentro del alveolo se producen cambios que han sido observados histológicamente tanto en estudios preclínicos como clínicos. Inmediatamente después de la extracción de un diente empieza la fase temprana de cicatrización que dura los primeros días, en la cual en el alveolo se forma un coágulo sanguíneo que durante los siguientes 5-20 días empieza a madurar transformándose en tejido de granulación. Posteriormente, durante la fase intermedia y hasta las semanas 8, disminuye la cantidad de tejido de granulación y aumenta la proliferación celular llegando a un pico de cantidad de osteoblastos a las 6-8 semanas, donde el tejido osteoide se mineraliza y se diferencia en hueso inmaduro, hasta terminar su maduración durante la fase tardía (semanas 12 - 24) y diferenciándose en hueso lamelar y cortical (Amler 1969; Cardaropoli y cols. 2003; Trombelli y cols. 2008).

b) Cambios extra-alveolares

Uno de los primeros estudios clínicos que evaluó los cambios extra-alveolares del alveolo post extracción, observó que tras la pérdida de los dientes se produce una reducción de la anchura de la cresta ósea de 3.8 mm a los 3 meses y hasta 6.1 mm a los 12 meses, lo que se corresponde con una reducción de aproximadamente el 50% de la anchura inicial (Schroop y cols. 2003). A

nivel vertical la reducción de la cresta ósea resultó menor con una media de 1.2 mm a los 12 meses. Además, se vio que la mayoría de los cambios (más del 60%) ocurrían durante los primeros 3 meses (Schroop y cols. 2003). Estos resultados han sido confirmados también en estudios preclínicos, en los que se observó que la mayor parte de la reabsorción ocurre en las primeras 8 semanas después de la pérdida dentaria, periodo caracterizado por un aumento de la actividad de los osteoclastos (Araujo & Lindhe 2005). Además, la reabsorción de la cresta ósea estaba más pronunciada en el aspecto vestibular, en comparación con la parte lingual. La explicación más plausible es que la tabla vestibular en los animales experimentales está compuesta prácticamente en su totalidad por el hueso fasciculado alveolar ("*bundle bone*") que es una estructura diente-dependiente relacionada con la presencia del ligamento periodontal. Esto conlleva que, una vez perdido el diente con su ligamento periodontal, disminuye la vascularización de la cresta ósea y aumenta la actividad osteoclástica con la consecuente mayor reabsorción de la cresta ósea vestibular (Araujo & Lindhe 2005; Araujo & Lindhe 2009). Una revisión sistemática publicada recientemente concluyó que durante los primeros 6-7 meses tras la pérdida o extracción dentaria se produce una reabsorción de la anchura de la cresta respectivamente de 3.79 mm a nivel coronal (29-63% a nivel horizontal), 1.24 mm a nivel vertical (11-22%) y en mesial y distal de 0.84 mm y 0.80 mm. Además, la mayor cantidad de cambios ocurría durante los primeros 3 meses, llegando a perder hasta un 32% en anchura. Solo después de 3 meses, se observó una reabsorción de 1.2 mm en vestibular, 0.9 mm en lingual y 0.5 mm en interproximal (mesial y distal). La menor reabsorción a nivel interproximal se relaciona con la presencia de los dientes vecinos que conservan su ligamento periodontal y con ello el hueso fasciculado alveolar en su alrededor (Tan y cols. 2012). Además, en esta revisión cuando se evaluaron los cambios entre 3 y 6-7 meses la reabsorción fue entre 0.9 - 3.6 mm por vestibular y 0.4 - 3 mm por lingual (Tan y cols. 2012). En humanos, tras la pérdida o extracción de los dientes se produce una reabsorción ósea en altura a nivel vestibular y lingual respectivamente de 1.67 mm y 2.03, y de 3.87 mm en anchura (Van der Weiden y cols. 2009).

c) Cambios extra-alveolares de los tejidos blandos

Al igual que las crestas óseas sufren alteraciones dimensionales, también en los tejidos blandos se producen cambios tras la pérdida dentaria, aunque estos son menos evidentes.

Se ha observado que los cambios dimensionales de los tejidos blandos tras la exodoncia o pérdida de los dientes están íntimamente relacionados con el fenotipo óseo. El espesor de la tabla vestibular en sujetos con fenotipo óseo fino es 0.7 mm de media, lo que traduce un mínimo espesor de los tejidos blandos en estos pacientes, mientras que en sujetos con fenotipo óseo grueso el espesor vestibular de los tejidos blandos y duros es respectivamente de 0.8 mm y 1.4 mm (Chappuis y cols. 2015). Tras la pérdida dentaria, mientras en los sujetos con fenotipo grueso, los tejidos blandos permanecen relativamente estables, en los sujetos con fenotipo fino se produce un espesamiento de los tejidos blandos. Esto se debe a que, en estos sujetos, la tabla ósea vestibular se reabsorbe rápidamente, lo que favorece el crecimiento y proliferación de los tejidos blandos, que ocupan el alveolo y, por lo tanto, aumentan su espesor. Sin embargo, en los fenotipos gruesos, se mantiene la estructura ósea y, por lo tanto, existen menos cambios a nivel de los tejidos blandos (Chappuis y cols. 2015).

Además, se observó que un tejido blando grueso presenta un mayor volumen de matriz extracelular y colágeno, así como una mayor vascularización, lo que permite una mayor respuesta inmunológica y de defensa para los tejidos. Es por esta razón que un mayor espesor de los tejidos blandos se acompaña por una mejor cicatrización ante un daño o trauma (Chappuis y cols. 2017).

En cuanto a los procesos de cicatrización de los tejidos blandos, lo que se observó fue que más del 51% de sus cambios se producen durante las primeras 2 semanas tras la pérdida dentaria, independientemente del fenotipo óseo. Además, a las 8 semanas de cicatrización, en los fenotipos finos el espesor de los tejidos blandos aumentó 4.8 mm, lo que se corresponde con siete veces más respecto al espesor inicial, mientras en los fenotipos gruesos el espesor de los tejidos blandos se quedó estable. También a nivel vertical los tejidos blandos sufren

alteraciones, pero lo que se produce es una reducción de la altura de respectivamente 1.6 mm en los fenotipos óseos finos y 1.4 mm en los gruesos (Chappuis y cols. 2015; Chappuis y cols. 2017).

Resultados similares se observaron después de 6 - 8 semanas en otro estudio clínico, no solo en términos de reducción de la altura de los tejidos blandos sino también en cuanto a ganancia de anchura en sentido horizontal. A nivel vertical se observó una reducción de la altura vestibular casi el doble respecto a la palatina, respectivamente de 0.98 mm y 0.56. Sin embargo, hubo un aumento de la anchura de los tejidos blandos en sentido horizontal de 0.13 mm a nivel palatino y entre 0.09 - 0.30 mm en vestibular (Farmer & Darby 2014).

El aumento de los tejidos blandos tras la pérdida dentaria se observó también en otro estudio clínico donde a los 6 meses hubo un aumento del espesor vestibular y lingual de respectivamente 0.4 mm y 0.5 mm, y a diferencia de los otros estudios mencionados previamente, se observó que el espesor de los tejidos blandos a nivel oclusal aumentó de 2.1 mm, con el completo cierre del alveolo (Iasella y cols. 2003).

Soluciones terapéuticas con implantes dentales

A pesar del aumento que puede ocurrir a nivel de los tejidos blandos, tras la pérdida dentaria, es frecuente encontrar crestas alveolares residuales atróficas como resultado de los cambios dimensionales en el hueso. En estas situaciones, la rehabilitación protésica con implantes dentales representa un tratamiento habitual cuya previsibilidad ha sido ampliamente descrita en la literatura, con una tasa de supervivencia superior al 96% después de 5 años y 95.2% después de 10 años (Jung y cols. 2008; Jung y cols. 2012). Resultados similares se observaron en otras revisiones sistemáticas, donde la tasa de supervivencia fue respectivamente de 95.6% y 93.1% después de 5 y 10 años, o incluso entre 92% y 95% después de 10 - 27 años (Moraschini y cols. 2015; Pjetursson y cols. 2012).

Aunque los resultados clínicos de los implantes dentales basados en tasas de supervivencia son en general muy altos, en las últimas dos décadas se ha avanzado mucho en el estudio de las superficies de implantes, desarrollando nuevos modelos de superficie que mejoren aún más la osteointegración y por lo tanto el éxito clínico de los implantes dentales, tanto a corto como a largo plazo. De hecho, se ha constatado, que la topografía macroscópica de los implantes tiene una íntima correlación con la diferenciación osteoblástica y con la mineralización ósea (Cooper y cols. 1999). Estudios in vitro han demostrado que en las superficies rugosas existe una mayor diferenciación de las células osteoblásticas, con una mayor actividad de la fosfatasa alcalina, junto con una mayor producción de osteocalcina y colágeno (Boyan y cols. 1998; Kieswetter y cols. 1996). Además, mientras la diferenciación osteoblástica es más pronunciada en las superficies rugosas, las células fibroblásticas son más activas en las superficies lisas (Brunski y cols. 2000).

A nivel microscópico distintas modificaciones de las superficies de los implantes mediante procesos de adición o sustracción (grabado ácido, chorreado de arena y láser) pueden cambiar las características fisicoquímicas de las superficies con cambios en su humectabilidad o su hidrofilia, o mediante la adición de iones metálicos, como la adición de fosfato cálcico, flúor u otros metales que confieran bioactividad a dichas superficies (Jansen y cols. 2003). Estas nuevas superficies bioactivas podrían mejorar el contacto entre el hueso y el implante y en consecuencia alcanzar una mejor osteointegración, especialmente en las fases iniciales de la cicatrización. Entre estas nuevas superficies bioactivas se ha investigado una nueva superficie formada a nivel microscópico por una capa mono molecular de multi-fosfonatos unida íntimamente a la superficie del titanio a través de enlaces covalentes (Viornery y cols. 2002). Dicha superficie es altamente hidrofílica, lo que confiere una alta estabilidad química y favorece su osteointegración. Además, estudios en vitro han demostrado que esta superficie imita uno de los componentes del hueso, la hidroxiapatita, aumentando la hidrofilia de la misma superficie del implante y en consecuencia una mayor actividad osteoblástica, lo que lleva a un mayor

contacto hueso-implante (Viornery y cols. 2002). Una de las ventajas de esta nueva superficie bioactiva es que es posible aplicarla en cualquier tipo de implante, independientemente de su macrodiseño.

Estudios preclínicos han observado una mayor osteointegración, no solo en las fases tempranas (entre 2 y 8 semanas) sino también a largo plazo (52 semanas) (Von Salis-Soglio y cols. 2014). Sin embargo, no solo es importante disponer de implantes dentales con propiedades óptimas de osteointegración, sino que es necesario disponer de una disponibilidad ósea suficiente como condición indispensable para la colocación óptima de los implantes y para garantizar un adecuado pronóstico a largo plazo, en términos de estética, función y salud peri-implantaria (Caneva y cols. 2010; Grunder y cols. 2005). De hecho, es relativamente común encontrar situaciones clínicas en las que el volumen óseo disponible no sea suficiente para la correcta colocación de los implantes dentales en su posición ideal. En dichos casos, la utilización de intervenciones de regeneración ósea tiene como objetivo aumentar la disponibilidad ósea y permitir la colocación de los implantes, devolviendo así función y estética a los pacientes (Benic & Hämmerle 2014).

Enfermedades peri-implantarias

A pesar de que los implantes dentales presentan una elevada tasa de supervivencia, estos pueden presentar complicaciones tempranas o tardías (Lang y cols. 2004; Quirynen y cols. 2014). Dentro de las complicaciones, las más frecuentes son las asociadas a fracasos de los componentes mecánicos de las restauraciones implanto-soportadas, como las fracturas de la prótesis o la pérdida de retención de las restauraciones. Sin embargo, las más relevantes, son las complicaciones biológicas como consecuencia de la afectación patológica de los tejidos periimplantarios (enfermedades peri-implantarias) (Pjetursson y cols. 2014; Pjetursson y cols. 2012; Jung y cols. 2008; Jung y cols. 2012).

Las enfermedades peri-implantarias, han sido clasificadas recientemente en el último Workshop Mundial de Periodoncia, identificándose dos entidades: mucositis periimplantaria y peri-implantitis (Caton y cols. 2018). A pesar que ambas mucositis periimplantaria y peri-implantitis son enfermedades de carácter inflamatorio asociadas a la placa bacteriana, se diferencian en que mientras que en la mucositis periimplantaria el infiltrado inflamatorio solo afecta a los tejidos blandos, en la peri-implantitis la inflamación de los tejidos blandos se acompaña por pérdida progresiva del hueso de soporte alrededor de la superficie del implante (Heitz-Mayfield & Salvi 2018; Schwarz y cols. 2018; Berglundh y cols. 2018; Renvert y cols. 2018; Ramanauskaite y cols. 2018).

La prevalencia de las enfermedades peri-implantarias varía según los estudios, entre 19-65% y 1-47% para mucositis periimplantaria y peri-implantitis, respectivamente, con una media de 43% para la mucositis periimplantaria y 22% para la peri-implantitis (Derks & Tomasi 2015). Las diferentes tasas de prevalencia reportadas en la literatura están influenciadas por distintos factores de riesgo, siendo en caso de la mucositis periimplantaria el acumulo de placa (pobre control OR= 1.9; muy pobre control OR= 2.9), tabaco (OR= 2.8 - 3.7) y radiación de cabeza y cuello (OR= 2.9) (Heitz-Mayfield & Salvi 2018), mientras que en la peri-implantitis, la historia previa de periodontitis (OR= 3- 9), acumulo de placa (pobre control OR= 14) y falta de terapia de mantenimiento. En cuanto a la diabetes Mellitus (OR=1.9) y el tabaco (OR= 2.01), a pesar que la asociación es alta, no hay suficiente evidencia para confirmar que se trate de verdaderos factores de riesgo de la peri-implantitis (Schwarz y cols. 2018).

Otros factores asociados al implante o a la localización donde se coloca el implante también han sido estudiados en cuanto a su posible papel en el inicio y en la progresión de la peri-implantitis, aunque los resultados no son concluyentes. Uno de estos factores es la superficie de los implantes, no sólo en cuanto a su macro diseño sino también en cuanto sus características microscópicas (Carrasco-Garcia y cols. 2019; Abrahamsson & Berglundh 2009; Renvert y cols. 2011; Rupp y cols. 2017; Saulaci & Schaller 2019; Asensio y cols. 2019; Jordana y cols. 2018).

Con respecto al diseño macroscópico de los implantes, a pesar de que algunos estudios afirman que las superficies rugosas están más relacionadas con una mayor progresión de la peri-implantitis, en comparación con las superficies lisa, otras revisiones sistemáticas concluyen que las diferencias no son significativas cuando se comparan ambas superficies con respecto a su tasa de supervivencia e incidencia de peri-implantitis (Saulaci & Schaller 2019; Jordana y cols. 2018). Las características microscópicas de las nuevas superficies de implantes antes mencionadas, no solo pueden mejorar los resultados clínicos al mejorar la superficie y la calidad del contacto hueso-implante, sino también podrían prevenir el desarrollo de las enfermedades peri-implantarias y dificultar su progresión (Asensio y cols. 2019).

La patogenia de las enfermedades peri-implantarias se ha estudiado fundamentalmente en modelos experimentales in vivo, tratando de comparar la iniciación y progresión de la peri-implantitis con la periodontitis, utilizando un modelo de peri-implantitis experimental en el perro Beagle, similar al modelo descrito hace décadas de periodontitis experimental (Lindhe y cols. 1992). En estos modelos experimentales, se facilita el acúmulo de placa bacteriana tanto alrededor de dientes, como de implantes al anudar ligaduras de algodón alrededor de su cuello y se estudian los cambios clínicos, radiográficos, microbiológicos e histológicos. Los resultados han mostrado una mayor inflamación en los tejidos blandos alrededor de los implantes, con respecto a la que había alrededor de los dientes, además de una destrucción más rápida de ambos tejidos blandos y duros peri-implantarios en dirección apical (Lindhe y cols. 1992). Estudios ulteriores utilizando similares modelos experimentales han demostrado que aunque existen similitudes en las características clínicas y microbiológicas entre ambas lesiones, las características histopatológicas de la periodontitis y de la peri-implantitis son diferentes, ya que la peri-implantitis presenta una progresión muchos más rápida, con un mayor infiltrado inflamatorio que provoca la extensión más apical de la destrucción ósea (Berglundh y cols. 1991; Schou y cols. 2002; Berglundh y cols. 2004; Hiyari y cols. 2018; Berglundh y cols. 2011). Estas diferencias pueden deberse a las diferencias estructurales entre ambos tejidos, siendo el

tejido conectivo peri-implantario en comparación con los dientes, deficiente en componentes celulares y vasculares, lo que podría traducirse en una mayor susceptibilidad al inicio de la enfermedad y con una mayor progresión de la lesión alrededor de los implantes (Sculean y cols. 2014; Ivanosky & Lee 2017).

Regeneración Ósea

Diferentes técnicas regenerativas han sido usadas a lo largo de los años para reconstruir crestas alveolares residuales, como la distracción alveolar, el uso de bloques de hueso o de factores de crecimiento. Sin embargo, la "Regeneración ósea guiada" (ROG) con la utilización combinada de injertos de reemplazo óseo y membranas barrera, es actualmente la técnica regenerativa más frecuentemente utilizada y documentada en la literatura científica (Hammerle & Jung 2003; Benic & Hammerle 2014; Sanz y cols. 2019). Dicha combinación mejora los resultados regenerativos de la utilización de los injertos o membranas solos, ya que el injerto óseo permite mantener mejor el espacio por debajo de la membrana y garantiza la formación de un coágulo sanguíneo estable, mientras que la membrana barrera asegura que la proliferación de las células al área regenerable son aquellas con potencial osteogénico (Benic & Hämmerle 2014; Sanz & Vignoletti 2015; Sanz y cols. 2019; Thoma y cols. 2019).

En función del momento en el cual se colocan los implantes dentales en relación al procedimiento ROG, se puede clasificar en: *técnicas simultáneas*, donde a la vez que se realiza la regeneración ósea se colocan los implantes en la misma intervención, y *técnicas diferidas*, cuando primero se realiza la ROG en una primera fase y tras un periodo de cicatrización y maduración ósea entre 2 y 6 meses, se colocan los implantes en una segunda intervención. Revisiones sistemáticas recientes reportan que la tasa de supervivencia de los implantes colocados en rebordes regenerados es superior al 95%, independientemente del tipo de abordaje. Además, se encontraron ganancias horizontales superiores a 4.2 mm cuando los implantes se colocan simultáneamente a la ROG, y de 3.9 mm en los procedimientos diferidos,

lo que demuestra que ambas técnicas son válidas cuando el objetivo es el aumento horizontal de la cresta alveolar residual (Sanz-Sanchez y cols. 2018; Sanz-Sanchez y cols. 2015).

Resultados similares han sido confirmados también en el último Workshop Europeo de Periodoncia sobre regeneración ósea, donde se demostró que la ROG es efectiva en la reconstrucción de las crestas óseas atróficas, independientemente del momento en el cual se colocan los implantes (Naenni y cols. 2019; Thoma y cols. 2019).

a) Regeneración Ósea Guiada. Uso de Sustitutos óseos

Los injertos óseos se pueden clasificar en función de su origen en: autoinjertos que proceden del propio individuo (hueso autólogo), aloinjertos que proceden de individuos diferentes al paciente, pero de la misma especie, xenoinjertos que proceden de una especie diferente a la del individuo, sintéticos y mixtos.

Los autoinjertos se consideran el "*gold estándar*", ya que son los únicos con capacidad osteogénica, osteoinductora y osteoconductora. Sin embargo, a pesar de su gran disponibilidad extraoral, su mayor desventaja es la poca disponibilidad intraoral, lo que supone una segunda cirugía para su obtención, lo cual puede conducir a complicaciones como lesiones en el sitio del donante, una mayor morbilidad, deformidades y cicatrices. Por estas razones, en la mayoría de los casos se utilizan actualmente injertos de reemplazo óseo o *sustitutos óseos*, bien sea en forma de hueso particulado o en bloque, (Benic & Hämmerle, 2014; Sanz & Vignoletti 2015; Sanz y cols. 2019; Haugen y cols. 2019).

Dentro de los sustitutos óseos, los xenoinjertos de origen bovina, a pesar de su baja tasa de reabsorción, presentan buena biocompatibilidad, osteoconducción e integración tisular, siendo considerados en la actualidad los sustitutos óseos de elección para la ROG de defectos óseos horizontales, sobre todo asociado al uso concomitante de membranas reabsorbibles (Hammerle y cols. 2008; Thoma y cols. 2019; Sanz-Sanchez y cols. 2018). Sin embargo, una válida alternativa a los xenoinjertos está representada por los injertos sintéticos, fundamentalmente los compuestos por biomateriales cerámicos, basados en distintas combinaciones de calcio (Ca) y

los fosfatos (PO4). Los más utilizados son la hidroxiapatita (HA) y los fosfatos cálcicos (TCP), solos o combinados. Su combinación permite modular su bioabsorbilidad, ya que en el caso de la HA ésta es lenta, mientras que la del TCP es rápida. Por esta razón el uso de materiales con diferentes proporciones de HA y TCP, lo que hoy en día conocemos como fosfato cálcico bifásico (BCP), permiten combinar las propiedades químicas asociadas a estos materiales, pudiendo de esta manera regular no solo su tasa de reabsorción, sino también sus propiedades bioactivas. El isómero β del TCP (β-TCP), se solubiliza en contacto con las células mesenquimales y se caracteriza por un pH fisiológico, micro porosidad homogénea, solubilidad elevada. Esta micro porosidad del material parece regular su degradación y de esta manera confiere un ambiente óptimo para la formación de nuevo hueso gracias a su capacidad osteoconductiva (LeGero y cols. 2003).

Uno de los estudios en vitro pioneros con diferentes "ratio" de HA/β-TCP ha mostrado que a mayor porcentaje de β-TCP se corresponde una mayor actividad osteoclástica y entonces una mayor degradación del material, pero al mismo tiempo una exagerada solubilidad no permite una activa reabsorción por parte de los osteoclastos, ya que la elevada liberación de iones Ca puede inhibir la misma actividad osteoclástica (Yamada y cols. 1997). Por estas razones, muchos estudios preclínicos y clínicos han comparados diferentes proporciones de HA/β-TCP para buscar el "ratio" ideal en las diferentes situaciones clínicas. El estudio preclínico de Nery y cols., en un modelo de perros "Beagle" se considera como uno de los primeros estudios donde se usaron diferentes "ratio" de HA/β-TCP y se observó que el β-TCP tiene un papel importante en la proliferación celular, así como en la revascularización y en la osteogénesis (Nery y cols. 1992). A partir de este estudio muchas investigaciones preclínicas y también estudios clínicos han empleado materiales a base de BCP tanto particulados como en bloque para tratar defectos óseos agudos y crónicos con morfologías diferentes, y todos confirman que el uso del β-TCP en la regeneración de los defectos óseo, facilita la formación de nuevo hueso gracias a su fácil capacidad de reabsorción en contacto con los fluidos biológicos, lo que proporciona su potencial

osteoconductivo a estos materiales (Von Arx y cols. 2001; Jensen y cols. 2006; Jensen y cols. 2007; Jensen y cols. 2009; Schwarz y cols. 2007; Tanuma y cols. 2013; Podaropoulos y cols. 2009; Trisi y cols. 2003; Van Assche y cols. 2013). Sin embargo, a pesar de las óptimas propiedades mencionadas anteriormente, todavía existe mucha controversia en cuanto al "ratio" ideal de HA/β-TCP.

b) Regeneración Ósea Guiada. Uso de Membranas Barrera

Uno de los principios básicos de la ROG se basa en el uso de membranas de barrera con el fin de excluir los tejidos blandos y permitir que las células con capacidad osteogénica procedentes del periostio, del endostio y de la médula ósea se diferencian en células formadoras de hueso promoviendo la regeneración de los tejidos (Dahlin y cols. 1988).

Las membranas usadas en regeneración ósea pueden clasificarse en reabsorbibles y no reabsorbibles. A pesar que ambas presentan características diferentes, estos dos tipos de membranas deben respetar algunas propiedades y requisitos comunes: biocompatibilidad, actividad biológica, capacidad de creación de espacio, porosidad / propiedades oclusivas, propiedades mecánicas, integración con los tejidos, tolerancia a la exposición, biodegradabilidad y fácil de manejar (Sanz y cols. 2019; Omar y cols.2019).

Las membranas no reabsorbibles que más se emplean en la ROG son las de politetrafluoroetileno expandido (e-PTFE), en muchas ocasiones reforzadas con una malla de titanio para garantizar una mayor resistencia y un menor hundimiento, lo que se traduce clínicamente en una mayor estabilidad del injerto subyacente. Su utilización está indicada en intervenciones de regeneración ósea cuando el objetivo es el aumento de hueso en sentido vertical, donde el mantenimiento del espacio es crítico para alcanzar el objetivo regenerativo. Sin embargo, cuando son utilizadas en técnicas ROG de aumento de hueso horizontal, varios ensayos clínicos han mostrado resultados similares al compararse con el uso de membranas reabsorbibles de colágeno nativo, con tasas de supervivencia de implantes colocados en el hueso regenerado superiores al 90% después de 12 años. Sin embargo, las membranas barrera, cuando

se exponen en el periodo postoperatorio, se ha demostrado que los resultados de la regeneración son inferiores, independientemente del tipo de membrana (Jung y cols. 2013; Macthei 2001; Naenni y cols. 2017; Friedmann y cols. 2002). La principal desventaja de las membranas no reabsorbibles es el alto riesgo de exposición temprana, lo que se traduce en un mayor riesgo de infecciones secundarias y dehiscencias de los tejidos blandos en la zona de intervención y un inferior resultado en cuanto al volumen de hueso regenerado (Simion y cols. 1994; Zitman y cols. 1997; Zitman y cols. 2001; Carpio y cols. 2000).

Las membranas reabsorbibles, por el contrario, no necesitan ser retiradas y presentan una menor morbilidad y una mejor respuesta a la exposición oral en comparación con las membranas no reabsorbibles. Dependiendo de su origen, las membranas reabsorbibles se pueden clasificar como naturales, fundamentalmente compuestas por colágeno (compuestas de colágeno tipo I y tipo III) o sintéticas (compuestas de polímeros sintéticos como ácido poliláctico o polietilenglicol) (Gentile y cols. 2011; Elgali y cols. 2017; Omar y cols. 2019).

Las membranas de colágeno son las membranas reabsorbibles que con más frecuencia se han usado en procedimientos de ROG. Éstas se integran fácilmente al ser el colágeno el componente principal del tejido conectivo y, por lo tanto, su uso se asocia con una baja morbilidad (Thoma y cols. 2019; Sanz y cols. 2019). Estudios preclínicos han demostrado que las membranas de colágeno tienen la capacidad per se de inducir la migración de células que expresan y secretan factores osteogénicos y angiogénicos (Schwarz y cols. 2006; Schwarz y cols. 2008; Sanz y cols. 2019). Sin embargo, debido a su falta de rigidez y rápida reabsorción (4-12 semanas) presentan claras limitaciones cuando se utilizan solas, sobre todo en el tratamiento de grandes defectos óseos, ya que, aunque pueden promover la formación de hueso regenerado (Guarneri y cols. 2017) tienden a colapsar en el defecto y el volumen de nuevo hueso es limitado (Benic & Hammerle 2014; Rothamel y cols. 2005; Owens & Yukna 2001; Zhao y cols. 2000; Schwarz y cols. 2006; Schwarz y cols. 2008).

Con el objetivo de aumentar la rigidez de estas membranas y extender su bioabsorbilidad se pueden tratar químicamente las membranas de colágeno mediante el proceso de entrecruzamiento (*crosslinking),* fundamentalmente a base de Formaldehido, Glutaraldehido, Difenilfosforilacida, Hexametilenedisocianato. Estas membranas presentan un mayor tiempo de reabsorción, son más rígidas, aunque parecen tener menos integración tisular y menos vascularización (Schwarz y cols. 2008; Friedman y cols. 2002; Friedman y cols. 2011). Sin embargo, una de sus mayores desventajas comparadas con las membranas de colágeno es la mayor tasa de exposición temprana, ya que las membranas cross-link se reabsorben por fagocitosis y degradación enzimática, resultando en una mayor respuesta inflamatoria (Rothamel y cols. 2005; Rothamel y cols. 2014; Bornstein y cols. 2007; Becker y cols. 2009). Cuando se exponen al medio bucal, suelen cicatrizar por segunda intención, con la epitelización tardía de la zona intervenida, aunque no suele existir interferencia significativa con los resultados de la regeneración (Benic & Hammerle 2014; Omar y cols. 2019; Friedman y cols. 2011; Moses y cols. 2005).

Las membranas reabsorbibles sintéticas están habitualmente compuestas por poliésteres alifáticos: poli (ácido láctico) (PLA), poli (ácido glicólico) (PGA), poli (épsilon-caprolactona) (PCL), poli (ácido hidroxilo Valerie), poli (ácido hidroxil butírico) y sus copolímeros. Las principales ventajas de estas membranas a base de polímeros sintéticos son su manejabilidad, biodegradación lenta y efecto barrera al estar controlada su porosidad. Sin embargo, su principal desventaja es su lenta bioabsorbilidad y su baja integración tisular, ya que su degradación intratisular puede generar una acidez que dificulta el proceso de cicatrización, aunque la mezcla de distintos copolímeros puede mejorar estos aspectos (grado de reabsorción, manejabilidad y medioambiente de cicatrización) (Gentile y cols. 2011; Omar y cols. 2019; Elgali y cols. 2017). A partir de los resultados histológicos, histomorfométricos y volumétricos de estudios in vivo en diferentes modelos animales, se ha observado que las membranas sintéticas se degradan más lentamente en comparación con las membranas de colágeno nativo, lo que permite mantener el

espacio para la regeneración más tiempo (Hoornaert y cols. 2016; Won y cols. 2016; Miller y cols. 1996). Las membranas sintéticas pueden fabricarse en capas con diferente grado de porosidad, combinando polímeros de ácido poliglicólico con citrato de acetiltributilo, permitiendo la integración tisular por su mayor porosidad externa y al mismo tiempo guiando la neoformación de hueso por su efecto barrera debido a su mínima porosidad en su capa interna (Gottlow y cols. 1994; Lundgren y cols. 1995; Araujo y cols. 1998). Modificando su composición polimérica se pueden modular los tiempos de biabsorbilidad, manteniendo su estructura íntegra incluso después de 6 semanas desde su implantación y degradándose por completo después de 6-12 meses, lo que permite mantener el espacio para la regeneración más tiempo (Gottlow y cols. 1994; Miller y cols. 1996; Lundgren y cols. 1994).

Además, al igual que las membranas cross-link, estas membranas en caso de exponerse al medio oral, tienen la capacidad de promover una cicatrización por segunda intención con la epitelización completa de la zona intervenida, sin alterar los procesos de regeneración (Matsumoto y cols. 2012; Schneider y cols. 2014). Gracias a estas propiedades las membranas sintéticas han sido usadas, tanto en estudios preclínicos como clínicos, no solo para regeneración ósea, sino para regeneración periodontal y para la cobertura de recesiones gingivales (Lundgren y cols. 1994; Lundgren y cols. 1997; Lundgren y cols. 1999; Mayfield y cols. 1998; Matsumoto y cols. 2012; Won y cols. 2016; Miller y cols.1996; Schneider y cols. 2014; Trombelli y cols. 1998).

Modelos Experimentales para evaluar las intervenciones de regeneración ósea

Los modelos experimentales preclínicos son las investigaciones de elección cuando se desarrolla una nueva tecnología o intervención con fines regenerativos. Los estudios in vitro sirven para estudiar la toxicidad, cito-compatibilidad y los mecanismos de diferenciación celular en respuesta frente a un nuevo material, pero no aportan informaciones sobre sus

capacidades regenerativas. Los estudios pre-clínicos in vivo son los tipos de estudios más frecuentemente utilizados en regeneración, ya que permiten evaluar histológicamente la respuesta tisular frente a las distintas tecnologías utilizadas con fines regenerativos, tanto en la reconstrucción de tejidos duros, como blandos en odontología (Pearce y cols. 2007). Estos modelos tratan de estudiar en un modelo animal el resultado de cicatrización más traducible al modelo humano, no sólo en cuanto a la respuesta tisular, sino también en relación con los periodos de cicatrización tras las distintas intervenciones, para de este modo poder extrapolar los resultados (Reinwald & Burr 2008; Pearce y cols. 2007; Draper 1994).

En estos estudios se han utilizado distintos modelos animales. Los cerdos presentan muchas similitudes en cuanto a las características del hueso con respecto a los humanos, sobre todo en términos de densidad ósea y de los tiempos de modelado y remodelado óseo, sin embargo, debido a su gran peso y al difícil manejo de estos animales, su uso no está muy extendido (Reinwald & Burr 2008; Pearce y cols. 2007).

Las ovejas y los perros son de manejo más fácil y representan los modelos que más se suelen elegir. Aunque las ovejas tienen una fisiología de sus huesos largos similares a los humanos adultos, el estudio de intervenciones intrabucales es muy complejo en estos animales debido a sus hábitos de deglución y digestión muy diferentes a los humanos (Reinwald & Burr 2008; Pearce y cols. 2007). Los perros son considerados como el animal experimental más favorable para estas intervenciones, ya que la composición del hueso es homogénea, aunque su densidad mineral es mayor que la humana. Sin embargo, la gran similitud entre los huesos corticales y esponjosos de los humanos y de los perros, no sólo en términos de fracción de agua, fracción orgánica, fracción inorgánica, sino también en cuanto a las respuestas de los tejidos, convierte este modelo en el de elección en la mayoría de las investigaciones, aunque su identificación como “animal de compañía”, hace que los comités éticos limiten su uso al mínimo posible (Reinwald & Burr 2008; Pearce y cols. 2007).

En cuanto a los tiempos de cicatrización, en términos de modelado y remodelado óseo, distintos autores sugieren que en los perros los tiempos son aproximadamente 4 veces mayores con respecto a los humanos, que son más lentos (Draper 1994). De manera similar, otras investigaciones confirman que el modelo en perros es el mejor también para estudiar la peri-implantitis inducidas por ligaduras, así como su evolución, ya que se vio que las configuraciones y el tamaño de los defectos de peri-implantitis en los perros son similares a los defectos en los humanos (Schwarz y cols. 2007; Golubovic y cols. 2012; Martins y cols. 2014). Por último, se tiene que considerar que el proceso de cicatrización puede cambiar entre los individuos de un estudio y también en el mismo individuo, dependiendo de las diferentes características anatómicas de las mandíbulas, pero también por el diferente potencial regenerativo y de cicatrización de cada individuo, e incluso de cada localización, factores estos que han sido constatados no solo en investigaciones preclínicas, sino también en ensayos clínicos (Sanz y cols. 2017; Schneider y cols. 2011).

Métodos de análisis de los tejidos blandos y duros

El análisis histológico se considera como la técnica "*gold estándar*" para evaluar la cicatrización y regeneración de tanto tejidos periodontales como periimplantarios. Las secciones histológicas permiten una evaluación detallada de los tejidos blandos y duros, pero esta información se refiere a una sola porción del defecto que en la mayoría de los casos tiene un grosor de 40-59 micras y, por lo tanto, no nos da una visión completa de la zona tratada. Además, a pesar que su uso es habitual en investigaciones preclínicas, su uso está muy limitado en la mayoría de los estudios clínicos por obvias razones éticas. Por ello, se han evaluado otras alternativas diagnósticas que nos permitan evaluar el comportamiento de los tejidos duros y blandos, tras las distintas intervenciones regenerativas. *Las radiografías convencionales* son una de las herramientas más empleadas en odontología, pero la imagen que nos proporcionan es bi-dimensional y no nos da una visión completa de la zona intervenida. Los métodos

radiográficos tridimensionales actuales, como *las tomografías craneales de haz cónico CBCT*, aportan una gran precisión, pero necesitan de exámenes seriados, lo que limita su uso por razones obvias de protección radiológica. Otros métodos muy empleados en los últimos años son las *mediciones extraorales directas* (modelos) o *intraorales* mediante el uso de sondas periodontales o calibres, aunque estos métodos carecen de precisión, con desviación estándares medias de alrededor de 1 mm (Chen y cols. 2008; Cardaropoli y cols. 2006; Ricci 2007; Osborn y cols.1990)

Recientemente, la *micro-tomografía computarizada (Micro-ct)* ha demostrado ser una herramienta complementaria muy útil y precisa para evaluar la cicatrización de los tejidos duros en la interfaz hueso-implante. En general, con el Micro-ct es posible cuantificar las proporciones de volumen óseo en el volumen total (BV/TV) (Ratio of bone volume/total volume), la superficie específica de hueso (BS/BV) (bone specific surface), la fracción de volumen óseo (BVF) (bone volume fraction), la densidad mineral del tejido (TMD) (tissue mineral density), la densidad mineral ósea (BMD) (bone mineral density), el espesor trabecular, y el porcentaje de contacto entre hueso e implante (BIC%) (bone to implant contact). Una de las ventajas del Micro-ct es que permite obtener las imágenes de manera rápida y sin dañar las muestras, aunque en muchas ocasiones es necesario seccionarlas antes de poderlas analizar *(Figura 1).*

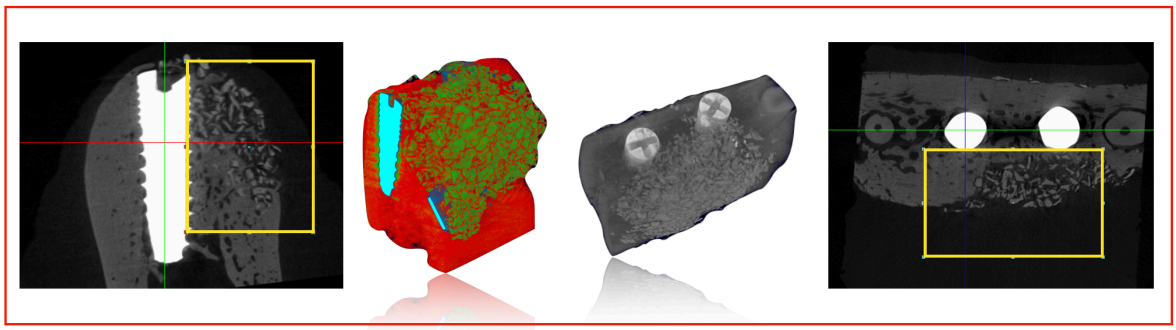


Figura 1. Representación gráfica de algunas secciones del Micro-ct usadas en un estudio de ROG. El rectángulo amarillo se corresponde con la región de interés

Además, a pesar que la histología se considera el "*gold estándar*" para evaluar el comportamiento de los tejidos, en un estudio preclínico en mini pig se observó que los análisis de los tejidos duros obtenidos con el Micro-ct no presentaban diferencias significativas en comparación con los resultados histológicos, y que además las mediciones presentaban una menor variabilidad cuando se repitieron más de dos veces en la misma muestra, lo que podría sugerir una ventaja añadida del Micro-ct (Bernhardt y cols. 2012).

Hasta el momento, la mayoría de las investigaciones publicadas en la literatura han utilizado el Micro-ct para cuantificar el hueso neo formado y la proporción del volumen óseo tras técnicas regenerativas con diferentes biomateriales y membranas, pero también para evaluar la densidad mineral del hueso y en caso de evaluar un nuevo sustituto óseo también cuantificar las partículas restantes de biomateriales (Finelle y cols. 2015; Nogueira y cols. 2010; Beck-Broichsitter y cols. 2015; Song y cols. 2014; Al-Askar y cols. 2016; Dahlin y cols. 2015; Beak y cols. 2016; Huang y cols. 2019; Matsumoto y cols. 2012; Won y cols. 2016; Khobragade y cols. 2015; Ramalingam y cols. 2016; Binsalah y cols. 2019; Hoornaert y cols. 2016; Al-Hazmi y cols. 2013; Al-hezaimi y cols. 2016; Antunes y cols. 2015; Di Raimondo y cols. 2020).

En dos estudios recientemente publicados sobre regeneración ósea y colocación simultánea de implantes, se ha utilizado el Micro-ct no solo para evaluar el volumen óseo regenerado, sino también los cambios lineales del contorno de los tejidos duros a diferentes alturas desde el hombro del implante, lo que significa una mayor precisión a la hora de interpretar los resultados de la regeneración (Thoma y cols. 2017; Di Raimondo y cols. 2020)

En los últimos cinco años, gracias a la precisión y los buenos resultados publicados en la literatura con el uso de Micro-ct, esta herramienta ha sido usada no solo en estudios de regeneración ósea, sino también para evaluar los niveles óseos alrededor de los implantes (Becker y cols. 2017), así como en peri-implantitis para calcular tridimensionalmente (360º) tanto la pérdida ósea peri-implantaria como la cantidad de hueso en contacto con el implante (BIC%) (Maglione y cols. 2019; Godoy-Gallardo y cols. 2016; Qian y cols. 2019; Varon-Shahar y cols. 2019; Hiyari y cols. 2018).

Los estudios de Micro-ct, sin embargo, presentan como gran desventaja que no pueden evaluar los tejidos blandos, lo que implica usar herramientas diferentes para evaluar ambos tejidos (blandos y duros), separadamente, o como se ha reportado en una reciente publicación mediante la combinación de imágenes DICOM procedentes del Micro-ct con imágenes STL obtenidas por escaneado digital directo o indirecto, y así estudiar ambos tejidos simultáneamente al superponer ambas imágenes (Sanz Martin y cols. 2018)

En los últimos años, se ha extendido también el uso de *Tecnologías Digitales* para la evaluación de cambios tisulares tras intervenciones regenerativas, sobre todo de tejidos blandos. Dentro de dichas tecnologías digitales, los escáneres ópticos permiten evaluar los cambios volumétricos y del contorno de los tejidos blandos que se producen después de diferentes procedimientos quirúrgicos. Dentro de los estudios pioneros de estas herramientas encontramos los estudios en vitro de Mehl y cols. y el de Windisch y cols. (Mehl y cols. 1997; Windisch y cols. 2007).

En el estudio de Mehl y cols. se observó que la precisión y la exactitud de la obtención de datos 3D depende de la inclinación de la superficie estudiada. Con más detalle, si la inclinación es

mayor de 60°, la precisión llega hasta 3µm y la exactitud hasta 6 µm, ambas sin llegar nunca a 10 µm (Mehl y cols. 1997). Resultados similares se observaron en el estudio de Windish y cols. donde los errores en las mediciones fueron siempre inferiores a 1 mm, con una resolución de hasta 20 micras, demostrando la eficacia y las ventajas de la tecnología digital (Windish y cols. 2007). Más recientemente, se compararon las mediciones obtenidas a partir de impresiones de alginato escaneadas con un escáner digital con las obtenidas a partir de un CBCT y se vio que a pesar que ambos son métodos válidos y reproducibles a nivel diagnóstico, el uso del escáner óptico es menos invasivo y más preciso (Wiranto y cols. 2013), lo que resulta en una mejor reproducibilidad y en una menor variabilidad intraoperador e interoperador (Schneider y cols. 2014). Dentro de las ventajas de las tecnologías digitales, una de las más importantes es que el objeto escaneado puede ser magnificado y estudiado desde diferentes angulaciones, permitiendo de esta manera realizar las mediciones varias veces y sin necesidad de la presencia física del paciente (Schneider y cols. 2014).

El uso de *escáneres intraorales* permite escanear el área de interés y convertir automáticamente las imágenes tridimensionales en archivos estereolitográficos (STL). Los escáneres extraorales precisan de la fabricación previa de un modelo de escayola procedente de una impresión intraoral convencional, que se escanea secundariamente y así se generan los correspondientes archivos STL.

A pesar que en la literatura se afirma que los escáneres intraorales parecen ser ligeramente más precisos, en comparación con los extraorales respectivamente de 20.7 - 33.35 µm versus 19.5 - 37 µm, a veces con los escáneres intraorales no es posible registrar correctamente zonas con márgenes profundos o incluso zonas que presentan sangrado (Sason y cols. 2018; Mangano y cols. 2017). Por el contrario, el uso de un escáner extraoral precisa que las impresiones sean registradas lo mejor posible con todos los detalles anatómicos y que los modelos estudiados no tengan burbujas o irregularidades en el vaciado, ya que podrían falsear los resultados.

Una vez obtenidos los archivos STL, para realizar el análisis del cambio de los tejidos blandos en las distintas fases de la investigación, es necesario efectuar la superposición (matching) de estos STL, mediante aplicaciones software específicas de análisis, como por ejemplo el SMOP (Swissmeda Software, Zurich, Switzerland) o el Geomagic (Geomagic Qualify 12; 3D Systems, Rock Hill, SC, USA), independientemente del tipo de escáner usado previamente *(Figura 2)*.

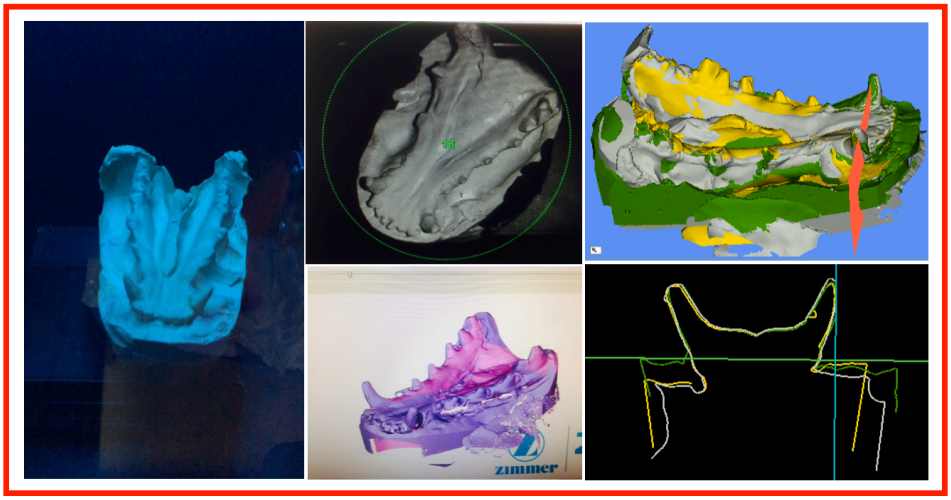


Figura 2. Representación gráfica del proceso de obtención y superposición de los archivos STL para el posterior análisis de los tejidos blandos.

Los escáneres ópticos han sido usados en los últimos años en distintos estudios preclínicos y clínicos, para medir los cambios volumétricos y lineales de los tejidos periodontales y periimplantares tras técnicas regenerativas, pero también tras cirugía de recubrimiento radicular, al igual que para evaluar el cambio del contorno de los tejidos desde la extracción de los dientes hasta su posterior rehabilitación (Fickl y cols. 2008; Fickl y cols. 2009; Schneider y cols. 2011; Strebel y cols. 2009; Thoma y cols. 2010; Gonzalez-Martin y cols. 2014; Rebele y cols. 2014; Sanz-Martin y cols. 2015; Schneider y cols. 2014; Jemt & Lekholm 2003; Jemt & Lekholm 2005; Henriksson y cols. 2004; Sanz-Martin y cols. 2016; Sanz-Martin y cols. 2017;

Zeltner y cols. 2017; Sanz-Martin y cols. 2018; Basler y cols. 2018; Rojo y cols. 2018; Di Raimondo y cols. 2020).

También ha sido usado recientemente en un estudio clínico en humanos para evaluar los cambios volumétricos de los tejidos blandos tras realizar técnicas de regeneración en peri-implantitis (Galarraga-Vinueza y cols. 2020). Aunque los escáneres ópticos son útiles para estudiar los tejidos blandos, estas herramientas presentan algunas desventajas. En primer lugar, está el coste de la tecnología, seguido por la necesidad de un largo proceso de aprendizaje, pero la desventaja mayor es que no aportan información en el comportamiento de los tejidos duros y por ello esta tecnología tiene siempre que acompañarse de evaluaciones radiográficas o histológicas.

A pesar de la elevada evidencia científica sobre la eficacia y la predictibilidad de la regeneración ósea guiada con diferentes biomateriales y membranas para el tratamiento de defectos horizontales, así como sobre la posible influencia de las superficies de los implantes en la prevención de la peri-implantitis y su progresión, no existe información comparativa del comportamiento de nuevos biomateriales sintéticos, tanto en sustitutos óseos como en material de membranas. Igualmente, no disponemos de información sobre el comportamiento de las superficies de implantes bioactivas frente a la agresión de la peri-implantitis experimental.

Además, en la mayoría de las investigaciones publicadas hasta el momento, el comportamiento de estos materiales ha sido evaluado clínicamente o histológicamente, sin disponer en la actualidad de información adecuada sobre el uso de metodologías tridimensionales alternativas, como el Micro-ct o las técnicas digitales para evaluar ganancias de volumen y/o cambios en los contornos de los maxilares tras técnicas regenerativas, lo que conlleva a buscar también cómo se comportan los tejidos blandos y duros en las distintas fases de la cicatrización, mediante análisis con las tecnologías digitales.

IV. JUSTIFICACIÓN

En base a estas deficiencias en cuanto a los métodos de evaluación tridimensional de los tejidos y específicamente en cuanto a la evaluación de los cambios volumétricos y de los contornos de ambos tejidos blandos y duros tras la realización de técnicas regenerativas (ROG) y tras la iniciación y progresión de enfermedades inflamatorias que cursan mediante la destrucción de los tejidos blandos y duros (peri-implantitis), hemos diseñado el presente proyecto de tesis doctoral que consta de tres estudios independientes, cada uno con una hipótesis específica, con el fin de validar estas nuevas tecnologías digitales y aumentar así los conocimientos actuales sobre estos biomateriales sintéticos utilizados en la ROG, al igual que sobre las nuevas superficies bioactivas de implantes para prevenir la progresión de la peri-implantitis.

Además, mientras el comportamiento de los tejidos duros en la peri-implantitis ha sido evaluado en otras investigaciones, la falta de datos acerca del comportamiento de los tejidos blandos en dicha enfermedad nos lleva a buscar también una nueva metodología para añadir informaciones adicionales en cuanto al comportamiento de los mismos para una mejor interpretación de los resultados no solo en las investigaciones preclínicas sino también en estudios clínicos futuros.

V. HIPÓTESIS

A partir de las consideraciones anteriormente descritas, la hipótesis general de la presente tesis es que la utilización de métodos de análisis tridimensionales (análisis digitales en archivos STL y análisis con Micro-ct a partir de imágenes DICOM) son capaces de evaluar tanto los cambios del contorno de los tejidos blandos, como los cambios volumétricos de los tejidos duros, no sólo en estudios experimentales que evalúan terapias regenerativas (estudios 1 y 2), sino también en estudios experimentales que evalúan la iniciación y la progresión de la peri-implantitis.

Si consideramos cada uno de los estudios de manera individual, mientras las hipótesis de los estudios 1 y 2 de ROG son similares ya que ambos estudios presentan el mismo diseño y protocolo, aunque evalúan materiales diferentes, las hipótesis del estudio 3 difieren debido a que se trata de un estudio de peri-implantitis.

De hecho, la hipótesis nula de los estudios 1 y 2 es que los tres enfoques quirúrgicos regenerativos obtienen resultados similares y que el grupo test no promueve una regeneración ósea superior, en comparación con el control positivo y negativo.

La hipótesis alternativa del estudio 1 es que los procedimientos de regeneración ósea guiada (grupo test y control positivo) promueven una mayor regeneración ósea con respecto al control negativo obteniendo así un mayor cambio del contorno de los tejidos blandos.

De manera similar, la hipótesis alternativa del estudio 2 es que los procedimientos de regeneración ósea guiada (grupo test y control positivo) promueven una mayor regeneración ósea con respecto al control negativo obteniendo así mayores cambios volumétricos y del contorno de los tejidos blandos y duros.

En cuanto al estudio 3 de peri-implantitis, la hipótesis nula es que la progresión de la peri-implantitis es similar en ambas superficies implantarias (test y control), y entonces los cambios del contorno de los tejidos blandos y los cambios volumétricos de los tejidos duros son

similares. La hipótesis alternativa sería que la superficie de los implantes con multi-fosfonatos (test) genera una mayor preservación del hueso periimplantario, con menores cambios volumétricos de los tejidos duros y menores cambios del contorno de los tejidos blandos.

VI. OBJETIVOS

Objetivo General

A partir de las hipótesis anteriormente formuladas, el objetivo general de la presente tesis es estudiar los procesos de cicatrización de los tejidos blandos y duros tras técnicas de regeneración ósea guiada y de peri-implantitis, en un modelo experimental de perros "Beagle", utilizando metodología digital y Micro-ct para evaluar de manera tridimensional tanto los cambios volumétricos y lineales de los tejidos duros, como los cambios del contorno de los tejidos blandos.

Objetivos Específicos

Los objetivos específicos han de ser divididos en función del tipo de estudio en cuestión (ROG y peri-implantitis), pero también en función de la metodología utilizada (análisis en archivos STL superpuestos y análisis en imágenes DICOM obtenidas con Micro-ct).

Si consideramos los estudios de regeneración ósea, los objetivos específicos son:

a) Evaluar el comportamiento de los tejidos blandos en términos de cambios lineales del contorno vestibular a diferentes alturas después de procedimientos de ROG con diferentes sustitutos óseos y membranas barrera, mediante superposición de archivos STL obtenidos a partir de impresiones de silicona
b) Comparar los resultados de la ROG entre los grupos en términos de aumento del contorno vestibular de los tejidos blandos y evaluar también a que altura de la cresta se obtuvieron más variaciones, si en la parte coronal, intermedia o apical.
c) Evaluar los cambios volumétricos en términos de aumento del volumen de tejidos duros mineralizados después de procedimientos de ROG con membranas barrera diferentes, mediante análisis con Micro-ct, y comparando los resultados entre los diferentes grupos, pero también evaluar los cambios lineales del contorno de los tejidos duros a diferentes

alturas, para evidenciar en que zona de la cresta ósea se obtuvo el mayor aumento de nuevo hueso.

Los objetivos específicos del estudio de peri-implantitis experimental son:

a) Evaluar el comportamiento de las dos superficies de implantes durante todas las fases de la peri-implantitis experimental, en términos de cambios lineales del contorno de los tejidos blandos tanto a nivel horizontal como a nivel vertical, mediante el análisis de modelos digitalizados (STL) superpuestos y comparando los resultados entre los grupos.
b) Proponer un nuevo método para aportar información sobre el comportamiento de los tejidos blandos en estudios experimentales in vivo de peri-implantitis, mediante la comparación de modelos estereolitográficos digitalizados (STL) superpuestos.
c) Evaluar si la superficie de los implantes con fosfonatos (grupo test) es superior con respecto a los implantes sin la superficie de fosfonatos (grupo control) en términos de cambios volumétricos de los tejidos duros (BIC%) y preservación del hueso peri-implantario, mediante el análisis de imágenes obtenidas con el Micro-ct.

VII. MATERIAL Y MÉTODOS. RESULTADOS

La descripción de los materiales y métodos, así como los resultados de las tres investigaciones han sido publicados como artículos científicos independientes y referenciados como sigue:

Artículo 1

Alveolar crests contour changes after guided bone regeneration using different biomaterials: an experimental in vivo investigation. Di Raimondo R, Sanz-Esporrin J, Pla R, Sanz-Martin I, Luengo F, Vignoletti F, Nuñez J, Sanz M. Clin Oral Investig (2020); 24 (7): 2351-2361

Artículo 2

Hard and soft tissue changes after guided bone regeneration using two different barrier membranes. An experimental in vivo investigation. Di Raimondo R, Sanz-Esporrin J, Sanz-Martin I, Pla R, Luengo F, Vignoletti F, Nuñez J, Sanz M. Clin Oral Investig. (2020) doi: 10.1007/s00784-020-03537-5

Artículo 3

Hard tissue volumetric and soft tissue contour linear changes at implants with different surface characteristics after experimentally induced peri-implantitis. An experimental in vivo investigation. Di Raimondo R, Sanz-Esporrin J, Sanz-Martin I, Vignoletti F, Nuñez J, Muñoz F, Haugen HJ, Sanz M. (Accepted with minor changes)

***Artículo 1**: Alveolar crests contour changes after guided bone regeneration using different biomaterials: an experimental in vivo investigation. Di Raimondo R, Sanz-Esporrin J, Pla R, Sanz-Martin I, Luengo F, Vignoletti F, Nuñez J, Sanz M. Clin Oral Investig (2020); 24 (7): 2351-2361*

Objetivo. Evaluar los cambios lineales del contorno vestibular de los tejidos blandos tras regeneración ósea guiada en defectos de dehiscencias peri-implantarias, con dos diferentes combinaciones de biomateriales

Material y métodos. Tras la hemisección y las extracciones de los dientes (M1M, Pm4M, Pm3D y Pm2) *(Imagen Suplementaria 1)*, se crearon tres defectos óseos en caja en cada hemi-mandíbula de los perros. Ocho semanas después se colocaron dos implantes en cada defecto y se realizó la regeneración ósea guiada en las dehiscencias vestibulares creadas previamente. Los defectos se aleatorizaron y se asignaron cada uno a un grupo diferente. i) Como test se usó la combinación de un sustituto óseo sintético a base de BCP formado por hidroxiapatita (HA 60%) y fosfato tricálcico (β-TCP 40%) cubierto por una membrana de colágeno entrecruzado; ii) como control positivo se usó la combinación de un xenoinjerto bovino (DBBM) cubierto por una membrana de colágeno nativo porcino; iii) el control negativo se dejó cicatrizar solo y sin ningún biomaterial *(Imagen Suplementaria 2)*. Estos procedimientos (extracciones, creación de defectos óseos, colocación de implantes y ROG) se repitieron en las hemi-mandíbulas contralaterales después de 8 semanas, y el sacrificio tuvo lugar después de otras 8 semanas, lo que se traduce en dos periodos de curación diferentes (8 y 16 semanas).

Resultados. Después de 8 semanas las diferencias entre los grupos no fueron significativas, aunque hubo una superioridad del grupo test y del control positivo, mientras después de 16 semanas de cicatrización el test y el control positivo obtuvieron una significativa mayor ganancia del contorno de los tejidos blandos en comparación con el control negativo.

Conclusiones. La ROG con la combinación de un sustituto óseo y una membrana barrera permite obtener un mayor aumento del contorno de los tejidos blandos. Además, a pesar que el "*gold estándar*" en regeneración ósea horizontal es combinar un xenoinjerto con una membrana de colágeno nativo, los resultados obtenidos sugieren que existen otras combinaciones de materiales, en cuanto a membranas y sustitutos óseos que permiten obtener resultados similares.

Clinical Oral Investigations (2020) 24:2351–2361
https://doi.org/10.1007/s00784-019-03092-8

ORIGINAL ARTICLE

Alveolar crest contour changes after guided bone regeneration using different biomaterials: an experimental in vivo investigation

R. Di Raimondo[1] · J. Sanz-Esporrín[2] · R. Plá[1] · I. Sanz-Martín[1] · F. Luengo[1] · F. Vignoletti[2] · J. Nuñez[1] · Mariano Sanz[1,2,3]

Received: 26 May 2019 / Accepted: 22 September 2019 / Published online: 9 November 2019

Abstract

Objective To evaluate the changes in alveolar contour after guided bone regeneration (GBR) with two different combinations of biomaterials in dehiscence defects around implants.

Material and methods Chronic alveolar ridge defects were created bilaterally in the mandible of eight Beagle dogs. Once implants were placed, three treatment groups were randomly allocated to each peri-implant dehiscence defect: (i) test group received a bone substitute composed of hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) covered by a cross-linked collagen membrane, (ii) positive control group with placement of deproteinized bovine bone mineral (DBBM) plus a porcine natural collagen membrane, and (iii) a negative control with no treatment. Two healing periods (8 and 16 weeks) were evaluated. Dental casts were optically scanned, the obtained files were uploaded into an image analysis software and superimposed to evaluate the linear changes.

Results In both healing periods, the gains in linear contours were higher in the test group and at the intermediate level (3 mm below the gingival margin). While at 8 weeks, no significant differences were found between the groups; at 16 weeks, the test and positive control groups demonstrated significant gains in contour compared with negative control.

Conclusions GBR using different biomaterials significantly increased the buccal contours of the alveolar crest when used at dehiscence defects around dental implants.

Clinical relevance Particulate highly porous synthetic bone substitute and a cross-linked collagen membrane demonstrated similar outcomes in terms of contour augmentation when compared to bovine xenograft (DBBM) and a collagen membrane.

Keywords Guided bone regeneration · Synthetic bone graft · Collagen membrane · Dental implant · Animal model · Prophilometric changes

MeSH Terms Bone Regeneration · Calcium Phosphates · Membranes · Biocompatible Materials · Dental Implants · Animal Model · Alveolar Bone Loss

Introduction

It is well established that irrespective of its cause, tooth loss will result in significant alterations in the alveolar process, in both the horizontal and vertical dimensions and hence impacting the hard and soft tissue contours [1, 2]. Recent systematic reviews have reported that the mean vertical loss at the buccal bone wall was 1.67 mm and the horizontal loss was 3.85 mm [3], with percentages of vertical and horizontal crestal bone resorption ranging between 11–22% and 29–63%, respectively. This high variability will be dependent on the cause of tooth loss. The main consequence of these changes is the compromise in bone availability for implant therapy and the direct impact on aesthetic contours of the maxillary profile.

This alveolar bone resorption can be compensated by different bone regenerative interventions, which have demonstrated efficacy for providing enough bone to allow ideal implant placement and for attaining aesthetic and functional

✉ Mariano Sanz
marsan@ucm.es

[1] Section of Graduate Periodontology, University Complutense, Madrid, Spain

[2] ETEP (Etiology and Therapy of Periodontal Diseases) Research Group, University Complutense, Madrid, Spain

[3] Facultad de Odontología, Plaza Ramón y Cajal s/n (Ciudad Universitaria), 28040 Madrid, Spain

implant supported restorations [4]. Among the different bone regenerative procedures, guided bone regeneration (GBR) using a bone replacement graft covered by a barrier membrane is currently the regenerative approach most widely used and documented in literature [5, 6].

Different biomaterials have been used as bone replacement grafts, such as autologous, allogenic, xenogeneic, and alloplastic materials [7, 8]. Although autologous grafts have been considered the standard of care for many years due to their osteogenic and osteoinductive properties, their use has important shortcomings, such as their fast resorption rate and the increased patient morbidity associated with its harvesting. On the other hand, xenografts composed of deproteinized bovine bone mineral (DBBM) exhibit excellent mechanical properties, high osteoconductivity, and slow bio-absorbability [9, 10].

Synthetic biomaterials, mainly ceramics, have also been widely used as bone replacement grafts. These are usually composed of biphasic calcium phosphates with different percentages of hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP). While β-TCP has a high turnover rate and rapid bio-absorbability, sintered HA may slow this process and allow for the needed of scaffolding effect and sustained space maintenance [8]. These synthetic biomaterials have shown promising results in experimental investigations [11, 12], although their predictive and clinical efficacy has not yet been demonstrated [10]. Recently, a new bone replacement graft made of biphasic calcium phosphate and hydroxyapatite (60% HA and 40% β-TCP) has reported enhanced wetting, high porosity, and excellent osteoconductivity [13].

Similar to bone replacement grafts, non bio-absorbable membranes were the state of the art when this regenerative concept was developed [14]. However, the frequent occurrence of exposures and its associated high morbidity have converted bio-absorbable membranes the barrier of choice for lateral bone augmentation [10]. These bio-absorbable membranes may undergo resorption either by enzymatic degradation (collagen membranes) or by hydrolysis (synthetic polymeric membranes), hence improving their tissue tolerance during wound healing, although these barrier membranes do not have the intrinsic capability for space maintenance and they always need to be used with a bone replacement graft to provide the needed scaffolding effect. This effect is dependent of the membrane bio-absorbability rate, which depends on its composition and the local environment conditions during healing (pH, temperature, etc.). Experimental investigations have reported that degradation of natural collagen membranes may start within 4 days to 4 weeks after membrane placement [15, 16]. This process may be extended by cross-linking the collagen composition of the membranes [17], although this usually requires chemical methods that may modify the collagen structure and cause undesirable local effects [18]. There is, however, no clear evidence on which is the ideal time for membrane degradation in order to maintain the barrier effect that attains optimal bone regeneration [8].

One controversial issue when assessing the efficacy of bone regenerative interventions, such as GBR, is how to evaluate the outcome, since the ideal histological results are restricted to experimental studies. While radiographic methods may seem ideal in light of the current 3D techniques such as cone beam computed tomography (CBCT) [19], the need of repeated examinations limits their use for obvious radiation protection measures. Direct bone measurements have been the most widely used [19]. These measurements require a secondary surgical intervention, which may coincide with the surgical intervention to place the implants; however, when bone augmentation is made in conjunction with implant placement, this second intervention is usually not needed. The advent of optical digital scanning has provided the potential to acquire precise and less invasive 3D stereolithographic (STL) images, which enables the superimposition of soft tissue contours and the comparison of both linear and volumetric changes, at both aesthetic and posterior zones [20]. The study of dimensional changes in alveolar ridges by means of STL image superimposition has been evaluated in both preclinical and clinical investigations [21–25]. It was therefore the objective of this experimental investigation to evaluate, by STL image superimposition, the efficacy of a lateral bone augmentation techniques based on the GBR principles, comparing a synthetic biphasic bone replacement graft plus a cross-linked collagen membrane with a positive control (DBBM plus a natural collagen membrane) and a negative control (no GBR).

Material and methods

Study design

This pre-clinical in vivo investigation was designed according to the modified ARRIVE guidelines [26] as a randomized controlled trial on large experimental animals (beagle dogs). The study was carried out at the Experimental Surgical Department of the Minimally Invasive Surgery Centre, Cáceres, Spain, after receiving approval from the Regional Ethics Committee for Animal Research. The digital analysis was performed in the Department of Periodontology, Faculty of Odontology of the University Complutense of Madrid, Spain.

Study population

Eight adult beagle dogs (6–7 years old) weighting between 10 and 20 kg were used for this investigation (four males and four females). The animals received a unique identification code through a subcutaneous chip (RFID). The research project was approved by the local ethics committee (CCMIJU Ref:

011/15). Animals were installed in individual kennels in a light/darkness cycle of 12:12 with a temperature of 21–22°. Food was based on hard animal food specific for this species and with free access to water. Animals were kept in groups in an area with natural light, fresh air, and regulated temperature. All animals were observed 2 weeks prior to the experiment to assess their general health status.

Surgical interventions

The surgical procedures have been previously described in an independent publication reporting the histological outcomes [13]. In brief, animals were sedated using propofol (2 mg/kg/i.v., Propovet, Abbott Laboratories, Kent, UK) and placed under general anesthesia with 2.5–4% of isoflurane (Isobavet, Schering-Plough, Madrid Spain), using a mechanical respirator during the entire surgery. Lidocaine 2% with epinephrine 1:100,000 (2% Xylocaine Dental, Dentsply, York, PA, USA) was further infiltrated locally.

The first surgery consisted on the extractions of P2, distal root of P3, mesial root of P4, and mesial root of M1 (Fig. 1a) and surgical creation of standardized osseous defects (10 × 10 × 5 mm) (Fig. 1b). The second surgery was carried out after 8 weeks of healing when these defects were chronified leading to a three wall knife edge alveolar crest. Once these defects were isolated after raising muco-periosteal flaps, two customized implants of 2.5 mm in diameter and 7–9 mm in length (Dentium® NR; Suwon, Korea) were placed on each of the three defect sites in each hemi-mandible. Implants were placed resulting in buccal dehiscence (Fig. 1c, d), which was measured with a periodontal probe UNC15 (Hu Friedy, Chicago, IL, USA) (Fig. 1e). These dehiscence defects were randomly treated in both the test and positive control groups with GBR or left untreated as the negative control group (Fig. 2a, b).

The same protocol of extractions was carried out in the contralateral hemi-mandible

- The test GBR intervention consisted on a synthetic bone replacement graft composed of 60% HA + 40% β-TCP (Osteon III®, Dentium, Suwon, Korea) and a crossed-linked collagen membrane (GENOSS® (Dentium, Suwon, Korea).
- The positive control GBR intervention consisted on deproteinized bovine bone mineral (DBBM) (BioOss® Geistlich, Wolhusen, Switzerland) and a natural porcine collagen membrane (BioGide® Geistlich, Wolhusen, Switzerland).

The third surgery carried out after a healing period of 8 weeks consisted on the same intervention described on the second surgery on the site where extractions were performed at surgery 2, thus allowing for two healing periods (2 and 4 months). After 8 weeks of healing from surgery 3, experimental animals were euthanized using a lethal dose of sodium Pentothal® (40–60 mg/kg/i.v., Dolethal, Vetoquinol, France), and mandibular specimens were retrieved for histologic analysis.

Stereolithography (STL) image acquisition and matching

Before the first surgical intervention, individual impression trays were fabricated for each dog, and before tooth extraction, mandibular impressions were obtained with a light/heavy silicon (Elite HD +, Zhermack spa, RO, Italy). The same procedure was repeated before each surgical intervention. From these impressions, a total of 24 cast models were poured in dental stone (Fujirock type 4, GC. Corp, Tokyo, Japan), which allowed for comparisons between baseline, 2 and 4 months of healing after the GBR intervention. Casts were digitized using a desktop 3D scanner (Zfx Evolution Scanner, Zimmer Dental, Bolzano, Italy) and STL files were obtained. Baseline, 8-week, and 16-week STL files were uploaded to a dedicated software® (SMOP, Swissmeda Software, Swissmeda AG, Zurich, Switzerland) for the process of matching (Fig. 3a). First matching was carried out using three clear and visible common references in both STLs, the baseline and follow-up casts, thus achieving a rough fit. Once this process was completed, further common reference points (no fewer than 10) were selected to achieve a "fine fit" where the software automatically superimposed the models using a series of mathematical algorithms [22].

Dimensional change measurements

Once the STL files were fully matched, a longitudinal slice that divided the ridge mesio-distally into two equal parts was selected. After that, a line coinciding with the axis of the tooth prior to its extraction was drawn in the middle of each cross-sectional image (vertical line). Then, perpendicular lines were drawn at three different levels, at 1, 3, and 5 mm from the most coronal aspect of the ridge, corresponding to the coronal, intermediate, and apical part of the defect. Linear measurements from the vertical line to the baseline and follow-up contours were calculated at the previously specified heights. To assess the changes in the ridge contour, the distance from the vertical line to the follow-up contour was subtracted to the distance from the vertical line to the baseline contour (Fig. 3b). All measurements were performed by a calibrated investigator (RDR).

Data analysis

Randomization of the interventions was performed using a computer-generated list that considers the side and position

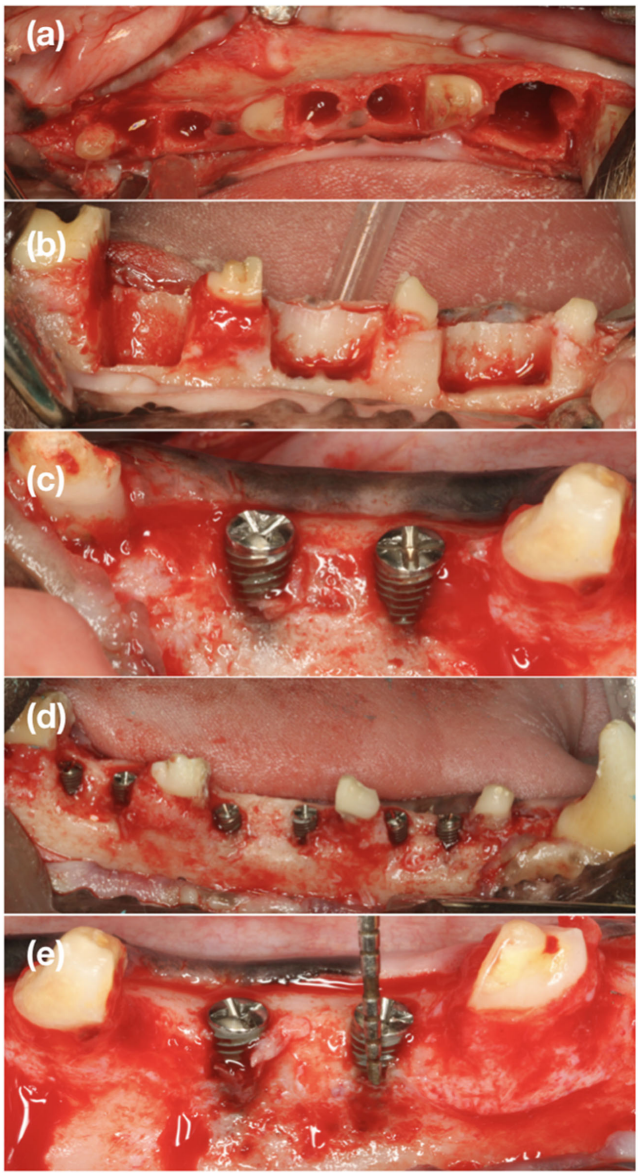


Fig. 1 Picture of the experimental surgeries. **a** Hemisection of P2, P3, P4, and M1 and extractions of P2, distal root of P3, mesial root of P4, and mesial root of M1. **b** Creation of bony defects after tooth extractions. **c** Implant placement with vestibular dehiscence in one defect. **d** Implant placement with vestibular dehiscence in the entire hemi-mandible. **e** Measurements of the dehiscence by periodontal probe

in the jaw (IBM SPSS Statistics® V20 JM.Domenech). Linear contour changes were calculated for each period (baseline–8 weeks/baseline–16 weeks) and expressed as means, standard deviation (SD), confidence intervals, and frequency distributions.

Shapiro-Wilk normality tests were performed to assess the data distribution. A general linear model was used to assess for multiple comparisons between absolute measurements of the primary outcome variable ("contour change"), considering the treatment group and healing time. ANOVA tests were used

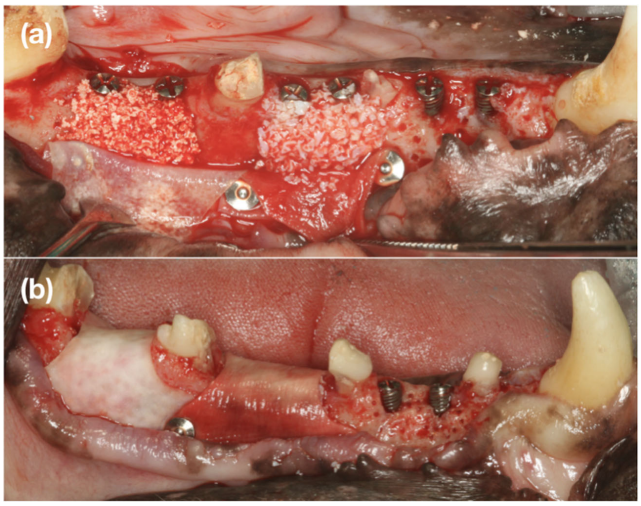


Fig. 2 Picture of GBR procedure. **a** Defect fill with both tested materials. **b** Membrane placement and fixation

for the intergroup comparisons, as well as for the differences depending on the height of the measurement (crestal, intermediate, and apical measurements at 1, 3, and 5 mm from the rim of the crest) and the position of the defect (mesial, central, or distal). Bonferroni corrections were performed for multiple comparisons. The alpha error was set at 0.05.

Intra-group comparisons were also performed to compare the contour changes within each treatment group between baseline-8 and baseline-16 weeks.

Results

Healing after the surgical interventions occurred uneventfully. One dog could not undergo final surgery due to an acute infection and the need of a hysterectomy. Another dog hemimandible could not be analyzed due to poor quality of the stone cast. Finally, 42 defects where analyzed, 14 for test group, 14 for positive control, 14 for negative control. Twenty-four defects were evaluated at 16 weeks of healing (8 test, 8 positive control and 8 negative control), while the remaining 18 defects were evaluated at 8 weeks of healing (6 test, 6 positive control and 6 negative control).

Intra-group comparisons

At 8 weeks, when comparing the width of the buccal contour changes in the three groups, a statistically significant increase was found only for the GBR procedures at 1 and 3 mm below the rim. Hence, at 1 mm below the rim of the crest, the mean lateral bone augmentation at test, positive control and negative control sites were of 0.77 mm (SD = 0.42) (p = 0.002), 0.84 mm (SD = 0.50) (p = 0.001), and 0.39 mm (SD = 0.55), respectively. At 3 mm below the crest, mean lateral bone augmentation at test, positive control and negative control sites were of 1.30 mm (SD = 0.76) (p = 0.004), 0.89 mm

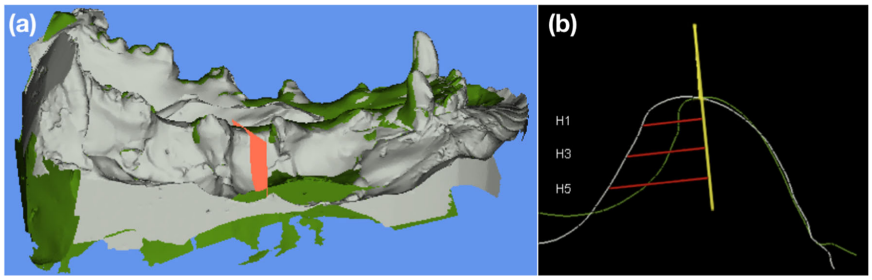


Fig. 3 Picture of the analysis. **a** Stl file matching. **b** Linear measurement between pre-GBR and post-GBR files; green line represents pre-GBR contour, while white line represents post-GBR contour. Red lines show the measurements performed in each part of the crest

(SD = 0.58) (p = 0.035), and 0.66 mm (SD = 1.31), respectively. Finally, at 5 mm, mean lateral bone augmentation at test, positive control and negative control sites were of 0.84 mm (SD = 0.81), 1.06 mm (SD = 1.03), and 0.48 mm (SD = 1.85), respectively. None of these lasts three measures demonstrated statistically significant differences.

At 16 weeks, only GBR procedures (test and positive control groups) obtained a statistically significant increase at the three different heights of the crest. At 1 mm below the crest, the mean lateral bone augmentation at test, positive control, and negative control sites were, respectively, 1.02 mm (SD = 0.74), 0.66 mm (SD = 0.90), − 0.07 mm (SD = 0.49). At 3 mm below the crest the mean lateral bone augmentation at test, positive control and negative control sites were 1.69 mm (SD = 0.62), 1.19 mm (SD = 0.62), and 0.40 mm (SD = 0.56), respectively. At 5 mm below the crest, the mean lateral bone augmentation at test, positive control, and negative control sites were 1.76 mm (SD = 0.93), 0.83 mm (SD = 0.52), and 0.22 mm (SD = 0.67), respectively (Table 1).

Inter-group comparisons

At 8 weeks, both GBR interventions achieved increased contour gains at the three different levels of the crest (1, 3, and 5 mm) compared to the negative control. One millimeter below the crest, the increase between the test and negative control groups was 0.44 mm (95% C.I. = − 0.36; 1.25), while the increase between the positive control and negative control groups was 0.51 mm (95% C.I. = − 0.29; 1.32). At 3 mm below the crest, the increase between the test and positive control groups versus the negative control was 0.64 mm (95% C.I. = − 0.82; 2.11) and 0.22 mm (95% C.I. = − 1.24; 1.69), respectively. Contour changes between the test and positive control, versus the negative control groups 5 mm below the crest, were 0.36 mm (95% C.I. = − 1.71; 2.45) and 0.58 mm (95% C.I. = − 1.60; 2.77), respectively. No one of these differences was statistically significant.

At 16 weeks, contour changes reached statistical significance when compared with the negative control group, although differences between the GBR groups were not statistically significant. One millimeter below the crest, contour changes between the test and negative control groups were 1.02 mm (95% C.I. = 0.07; 1.98) (p = 0.032) while the contour changes between the positive control and negative control groups were 0.66 mm (95% C.I. = − 0.28; 1.62) (p = 0.247). At 3 mm below the crest, contour changes between the test and positive control groups versus the negative control were 1.29 mm (95% C.I. = 0.51; 2.07) (p = 0.001) and 0.79 mm (95% C.I. = 0.01; 1.57) (p = 0.044), respectively. Contour changes between the test and positive control, versus the negative control groups 5 mm below the crest, were 1.54 mm (95% C.I. = 0.45; 2.63) (p = 0.005) and 0.61 mm (95% C.I. = − 0.47; 1.70) (p = 0.462), respectively (Table 2).

Contour changes from baseline to both healing periods (8 and 16 weeks) depending on the defect position

Although the contour changes were higher for the mesial defects at 1 and 3 mm below the crest, differences in contour changes among the defects of the different treatment groups were not statistically significant (Table 3).

Contour changes were also assessed depending on the apico-coronal level from the rim of the crest: the most coronal level (1 mm from the rim), intermediate (3 mm), and apical (5 mm). Considering all the sample together, mean changes were 0.59 mm (SD = 0.71), 1.03 mm (SD = 0.84), and 0.88 mm (SD = 1.09), respectively. These differences were not statistically significant.

However, when the healing periods were analyzed separately, the mean changes in crest profile after 8 weeks at the intermediate, apical, and coronal level were 0.95 mm (SD = 0.92), 0.78 mm (SD = 1.26), and 0.65 mm (SD = 0.54), respectively (Fig. 4a). After 16 weeks, these changes at the

Table 1 Contour changes (mm) from baseline to 8 weeks and baseline to 16 weeks (BSL-8W) (BSL-16 W). ***$p < 0.05$. Intragroup comparison (mean (SD))

		BSL 8W	Post GBR	Diff BSL-8W	P	BSL 16W	Post GBR 16W	Diff BSL-16W	P
TEST	1 mm	2.33 (0.76)	3.10 (0.64)	0.77 (0.42)	*** 0.002	2.67 (0.31)	3.69 (0.70)	1.02 (0.74)	0.001
	3 mm	2.75 (0.52)	4.05 (1.14)	1.30 (0.76)	*** 0.004	3.43 (1.29)	5.12 (1.23)	1.69 (0.60)	0.000
	5 mm	4.35 (1.16)	5.20 (1.29)	0.84 (0.81)	0.14	4.54 (2.33)	6.31 (1.84)	1.77 (0.93)	0.000
POSITIVE CONTROL	1 mm	2.41 (0.43)	3.26 (0.66)	0.84 (0.50)	*** 0.001	2.41 (0.69)	3.07 (0.82)	0.66 (0.90)	0.018
	3 mm	2.99 (1.01)	3.88 (0.92)	0.89 (0.58)	*** 0.035	3.57 (0.82)	4.76 (0.71)	1.19 (0.62)	0.000
	5 mm	3.13 (1.02)	4.20 (0.69)	1.06 (1.03)	0.094	4.45 (1.25)	5.28 (1.07)	0.83 (0.52)	0.008
NEGATIVE CONTROL	1 mm	2.02 (0.91)	2.42 (1.20)	0.39 (0.55)	0.068	2.89 (0.81)	2.88 (0.78)	− 0.07 (0.49)	0.979
	3 mm	3.16 (0.41)	3.83 (1.10)	0.66 (1.31)	0.105	3.34 (0.66)	3.74 (0.71)	0.40 (0.56)	0.071
	5 mm	4.76 (1.16)	5.24 (1.46)	0.48 (1.85)	0.390	3.93 (0.87)	4.15 (0.70)	0.22 (0.67)	0.474

Table 2 Contour changes (mm) at 8 weeks and 16 weeks between groups corresponding to the three different crestal levels (1 mm, 3 mm, 5 mm). Δdifferences; ***$p < 0.05$. Intergroup comparison (mean difference (95% CI))

Healing	Measurements	Mean width test	Mean width control +	Mean width control –	Δ Mean T/C+ (mm)	Confidence interval 95%	*P*	Δ Mean T/C– (mm)	Confidence interval 95%	*P*	Δ Mean C/C+ (mm)	Confidence interval 95%	*P*
8 weeks	1 mm	0.77 (0.42)	0.84 (0.50)	0.39 (0.55)	− 0.07	− 0.88; 0.73	1.000	0.38	− 0.36; 1.25	0.485	0.45	− 0.29; 1.32	0.319
8 weeks	3 mm	1.30 (0.76)	0.89 (0.58)	0.66 (1.31)	0.41	− 1.04; 1.88	1.000	0.64	− 0.82; 2.11	0.765	0.22	− 1.24; 1.69	1.000
8 weeks	5 mm	0.84 (0.81)	1.06 (1.03)	0.48 (1.85)	− 0.21	− 2.40; 1.96	1.000	0.36	− 1.71; 2.45	1.000	0.58	− 1.60; 2.77	1.000
16 weeks	1 mm	1.02 (0.74)	0.66 (0.90)	− 0.07 (0.49)	0.35	− 0.59; 1.31	1.000	1.02	0.07; 1.98	*** 0.032	0.66	− 0.28; 1.62	0.247
16 weeks	3 mm	1.69 (0.60)	1.19 (0.62)	0.40 (0.56)	0.49	− 0.28; 1.27	0.337	1.29	0.51; 2.07	*** 0.001	0.79	0.01; 1.57	**** 0.044
16 weeks	5 mm	1.77 (0.93)	0.83 (0.52)	0.22 (0.67)	0.93	− 0.11; 1.97	0.092	1.54	0.45; 2.63	*** 0.005	0.61	− 0.47; 1.70	0.462

intermediate, apical, and coronal level were 1.09 mm (SD = 0.78), 0.97 mm (SD = 0.95), and 0.55 mm (SD = 0.82), respectively (Fig. 4b).

Discussion

This experimental study evaluated the contour changes when comparing two lateral bone augmentation interventions based on the principles of guided bone regeneration in conjunction with implant placement. The treatment groups consisted of a test group using a synthetic bone replacement graft composed of 60% HA + 40% β-TCP together with a cross-linked collagen membrane, a positive control group using as xenogeneic bone replacement graft (DBBM) and a native collagen barrier membrane and finally a negative control group without any regenerative materials. At 8 weeks, there were no significant differences among the tested interventions. After 16 weeks of healing, significantly greater gain in ridge contours was found in both the test and positive control groups when compared to the negative control. Although the gains in buccal crestal contours were superior in the test group when compared with the positive control group, these differences were not statistically significant.

These results may be explained by the different behavior of the biomaterials and membranes used due to their inherent biologic properties. When used as bone replacement grafts, histological studies have demonstrated the different resorption rates of HA and β-TCP, with HA demonstrating slower resorption and hence, greater scaffolding effect [27, 28]. These findings were also corroborated in a preclinical study in which GBR procedures were performed with these two bone substitutes. It was demonstrated that after 3 months, there was significant resorption of β-TCP and complete substitution with new bone after 24 months, while DBBM particles remained unresorbed throughout 24 months [29]. Moreover, in a recent clinical study, it was reported that 11 years after sinus floor augmentation, DBBM particles were identified integrated with the regenerated bone [30].

In an experimental study in minipigs, healing dynamic and histometric differences were assessed between β-TCP, DBBM and an autograft when used in GBR procedures with a non-resorbable e-PTFE membrane. Authors concluded that at the initial healing stages, newly formed bone was present in higher amounts in the autograft when compared to the β-TCP and DBBM. Nevertheless, after 8 weeks of healing, the percentage of newly formed bone was comparable between β-TCP and the autograft, being statistically higher than DBBM. At the conclusion of the study autograft and β-TCP were almost totally substituted by newly formed bone, whereas DBBM remained stable [31]. In the present study, no superiority was observed by DBBM which may be explained by the use of a different

Table 3 Changes (mm) after lateral bone augmentation regarding both healing periods (8 and 16 weeks) based on the position of the defect (mesial, central, or distal) in the three treatment groups (test, positive control and negative control) (mean (SD))

		Mesial defect	Central defect	Distal defect	*p*
Diff BSL-8W	1 mm	0.84 (0.57)	0.50 (0.44)	0.66 (0.52)	0.550
	3 mm	1.13 (1.36)	0.71 (a 64)	1.01 (0.73)	0.745
	5 mm	0.67 (1.96)	0.79 (1.09)	0.87 (0.92)	0.970
Diff BSL-16W	1 mm	0.76 (0.91)	0.43 (0.81)	0.47 (0.81)	0.714
	3 mm	1.29 (0.89)	1.15 (0.90)	0.84 (0.55)	0.523
	5 mm	0.97 (0.91)	1.31 (1.21)	0.63 (0.65)	0.440

barrier membrane which may have affected the behavior of the biomaterial.

In this study, the rate of HA/β-TCP was 60/40 what may have decreased the biomaterial resorption but maintaining the high porosity and osteoconductivity demonstrated by β-TCP. These properties have been demonstrated when DBBM is used as a bone replacement graft [32]. In fact, in this investigation, the use of both bone replacement grafts demonstrated a similar performance in regard to the hard tissue gains when the histological outcomes were reported [13]. Randomized clinical trials have also failed to find differences when comparing DBBM and β-TCP for the treatment of peri-implant dehiscence defects [7]. However, when evaluating the augmented bone thickness at 0, 1, and 2 mm apical to the implant shoulder, the histological results reported significantly greater gains for HA/β-TCP+ a cross-linked collagen membrane when compared to DBBM+ a natural collagen membrane [13]. These findings were attributed to the utilization of a cross-linked collagen membrane in the test group which caused the formation of a band of periosteum-like tissue. The contour changes in this study corroborate these histological outcomes, which may be more attributable to the different membranes used, rather than the bone replacement graft. The study of the behavior of cross-linked collagen membranes has shown that they may retain their structure during a period of 16 weeks [33], while native collagen membrane has faster resorption rates (approximately 8 weeks) [18, 34]. This prolonged barrier function may have provided with better space maintenance, which may explain the greater contour increase in the test group at 16 weeks, whereas at 8 weeks when both membranes were not completely biodegraded, there were no differences. Clinically, it appears that the method of cross linking determines the behavior of the membrane since there are reports of improved clinical outcomes when using ribose cross-linked collagen membranes compared against native collagen membranes [35], while other studies have reported soft tissue complications when using cross-linked collagen membrane and inferior outcomes [18, 36, 37].

The methodology used in this preclinical investigation allowed the evaluation of the changes in ridge contours in a non-invasive manner granting for multiple comparisons over time [25, 38–41]. The use of digital STL analysis allows to study not only the possible hard tissue gains,

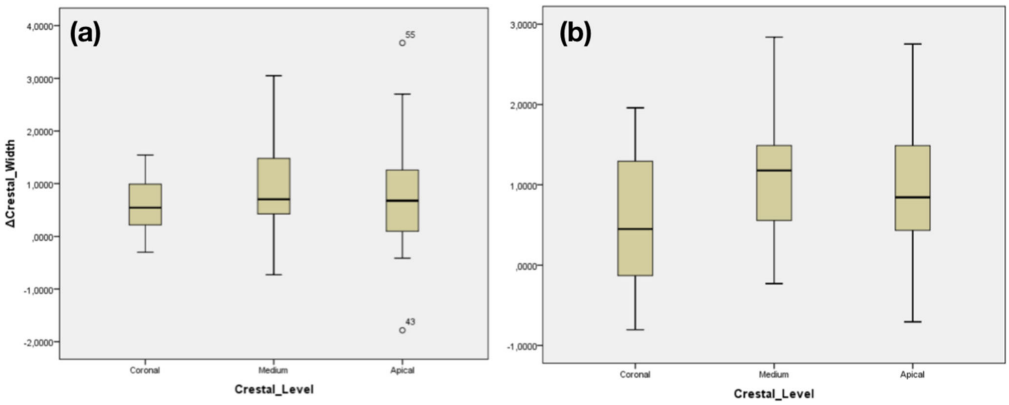


Fig. 4 Picture of the box plots representing the changes that took place after both healing periods. **a** Box plot representing contour changes that took place after 8 weeks at 1-, 3-, and 5-mm levels. **b** Box plot representing contour changes that took place after 16 weeks at 1-, 3-, and 5-mm levels

but also the soft tissue changes after the use of bone augmentation procedures. Recently, an experimental investigation using a similar methodology reported that the combination of a bone replacement graft plus a collagen membrane led to a greater buccal volume gain when compared to membrane and biomaterial alone, in staged augmentation procedures, although none of the regenerative interventions was able to recover the volume lost after defect creation [23]. In spite of the differences in study design (staged versus simultaneous augmentation), these results are in line with the findings of the present investigation in which after both healing periods (8 and 16 weeks), crestal contours were greater in the regenerative groups when compared to the negative control.

Interestingly, when the contour changes in the most apical levels were evaluated in the present investigation, no significant differences were observed at the 8-week healing period; however, statistical significant differences were observed in the apical level of the crest at the 16 healing week period. These findings are challenging to explain taking the lack of interventions in the period where changes occurred. The apical portions of the crest are the most sensitive areas to register taking that alveolar mucosa is frequently encountered which can vary according to pressure, and therefore, inaccurate readings may have been introduced. It is thus possible that an apical displacement of the biomaterial occurred throughout the last 8 weeks of the healing and influenced the contour changes.

The present data should be interpreted with caution due to the experimental in vivo nature of this investigation which used different membranes that prevented a clear comparison of the effect of the bone substitute material. Moreover, the present investigation only reported the changes in tissue contour which provides insufficient information to clearly understand the tissue dynamics since the hard tissue information is lacking. Nevertheless, the present investigation provided with information on the effect of different regenerative strategies on the soft tissue contours allowing to establish clear relationships with the observed hard tissue changes.

Conclusions

Within the limitations of the present experimental investigation, it can be concluded that test (HA + β-TCP) and positive control group (HA) obtained statistically significant more volume gain after lateral bone augmentation compared with negative control after 16 weeks with no significant differences between two regenerative approaches.

Acknowledgments The authors acknowledge professor Ui-Won Jung for the active involvement in the surgical procedure. We also thank the veterinary doctors, Maria Carmen Calles-Vázquez and Elena Abellán, as well as the staff from the Minimally Invasive Surgery Centre, Cáceres, Spain, who so effectively took care of the experimental animals used in this investigation.

Funding information This work was partially supported through a research contract between the University Complutense of Madrid and Dentium Implants, Suwon (Korea). Support was also obtained from the ETEP (Etiology and therapeutics in Periodontal Diseases) Research Group at the Faculty of Odontology, University Complutense of Madrid (Spain).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article contains data from an experimental study with animals performed at the Experimental Surgical Department of the Minimally Invasive Surgery Centre in Cáceres (Spain) after receiving approval from the Regional Ethics Committee for Animal Research (CCMIJU Reference 011/15). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent For this type of study, formal consent is not required.

References

1. Schropp L, Kostopoulos L, Wenzel A, Isidor F (2005) Clinical and radiographic performance of delayed-immediate single-tooth implant placement associated with peri-implant bone defects. A 2-year prospective, controlled, randomized follow-up report. J Clin Periodontol 32(Suppl. 5):480–487
2. Fickl S, Zuhr O, Wachtel H, Stappert CF, Stein JM, Hurzeler MB (2008) Dimensional changes of the alveolar ridge contour after different socket preservation techniques. J Clin Periodontol 35(Suppl. 10):906–913
3. Van der Weijden F, Dell'Acqua F, Slot DE (2009) Alveolar bone dimensional changes of post-extraction sockets in humans: a systematic review. J Clin Periodontol 36(Suppl. 12):1048–1058
4. Benic GI, Hammerle CH (2014) Horizontal bone augmentation by means of guided bone regeneration. Periodontol 2000 66(Suppl 1): 13–40
5. Hammerle CH, Jung RE (2003) Bone augmentation by means of barrier membranes. Periodontol 2000(33):36–53
6. Von Arx T, Cochran DL, Hermann JS, Schenk RK, Higginbottom FL, Buser D (2001) Lateral ridge augmentation and implant placement: an experimental study evaluating implant osseointegration in different augmentation materials in the canine mandible. Int J Oral Maxillofac Implants 16:3
7. Van Assche N, Michels S, Naert I, Quirynen M (2013) Randomized controlled trial to compare two bone substitutes in the treatment of bony dehiscences. Clin Implant Dent Relat Res 15(Suppl 4):558–568
8. Sanz M, Vignoletti F (2016) Key aspects on the use of bone substitutes for bone regeneration of edentulous ridges. Dent Mater 31(Suppl 6):640–647
9. Araujo M, Linder E, Lindhe J (2009) Effect of a xenograft on early bone formation in extraction sockets: an experimental study in dog. Clin Oral Implants Res 20(1):1–6

10. Sanz-Sanchez I, Ortiz-Vigon A, Sanz-Martin I, Figuero E, Sanz M (2015) Effectiveness of Lateral Bone Augmentation on the Alveolar Crest Dimension: A Systematic Review and Meta-analysis. J Dent Res 94(Suppl 9):128S–142S
11. Tanuma Y, Matsui K, Kawai T, Matsui A, Suzuki O, Kamakura S (2013) Comparison of bone regeneration between octacalcium phosphate/collagen composite and beta-tricalcium phosphate in canine calvarial defect. Oral Surg Oral Med Oral Pathol Oral Radiol 115(Suppl 1):9–17
12. Trisi P, Rao W, Rebaudi A, Fiore P (2003) Histologic effect of pure-phase beta-tricalcium phosphate on bone regeneration in human artificial jawbone defects. Int J Periodontics Restorative Dent 23(Suppl 1):69–77
13. Jung UW, Cha JK, Vignoletti F, Nuñez J, Sanz-Esporrin J, Sanz M (2017) Simultaneous lateral bone augmentation and implant placement using a particular synthetic bone substitute around chronic peri-implant dehiscence defects in dogs. J Clin Periodontol 44(11):1172–1180
14. Dahlin C, Linde A, Gottlow J, Nyman S (1988) Healing of bone defects by guided tissue regeneration. Plast Reconstr Surg 81:672–676
15. Zhao S, Pinholt EM, Madsen JE, Donath K (2000) Histological evaluation of different biode- gradable and nonbiodegradable membranes im- planted sub-cutaneously in rats. J Craniomaxillofac Surg 28:116–122
16. Owens KW, Yukna RA (2001) Collagen membrane resorption in dogs: a comparative study. Implant Dent 10:49–56
17. Paul BF, Mellonig JT, Towle HJ III, Gray JL (1992) Use of a collagen barrier to enhance healing in human periodontal furcation defects. Int J Periodontics Restorative Dent 12:123–131
18. Schwarz F, Rothamel D, Herten M, Wustefeld M, Sager M, Ferrari D (2008) Immunohistochemical characterization of guided bone regeneration at a dehiscence-type defect using different barrier membranes: an experimental study in dogs. Clin Oral Implants Res 19(Suppl 4):402–415
19. Chen LC, Lundgren T, Hallstrom H, Cherel F (2008) Comparison of different methods of assessing alveolar ridge dimensions prior to dental implant placement. J Periodontol 79(Suppl 3):401–405
20. Hammerle CHF, Cordaro L, Van Assche N, Benic GI, Bornstein M, Gamper F, Gotfredsen K, Harris D, Hurzeler M, Jacobs R, Kapos T, Kohal RJ, Patzelt SBM, Sailer I, Tahmaseb A, Vercruyssen M, Wismeijer D (2015) Digital technologies to support planning, treatment, and fabrication processes and outcome assessments in implant dentistry. Summary and consensus statements. The 4th EAO consensus conference 2015. Clin Oral Implant Res 26(Suppl 11): 97–101
21. Fickl S, Schneider D, Zuhr O, Hinze M, Ender A, Jung RE (2009) Dimensional changes of the ridge contour after socket preservation and buccal overbuilding: an animal study. J Clin Periodontol 36(Suppl 5):442–448
22. Sanz Martin I, Vignoletti F, Nuñez J, Permuy M, Muñoz F, Sanz-Esporrin J, Fierravanti L, Shapira L, Sanz M (2017) Hard and soft tissue integration of immediate and delayed implants with a modified coronal macro design: Histological, micro CT and volumetric soft tissue changes from a pre-clinical in vivo study. J Clin Periodontol 44(8):842–853
23. Sanz Martin I, Ferrantino L, Vignoletti F, Nuñez J, Baldini N, Duvina M, Alcaraz J, Sanz M (2018) Contour changes after guided bone regeneration of large non-contained mandibular buccal bone defects using deproteinized bovine bone mineral and a porcine-derived collagen membrane: an experimental in vivo investigation. Clin Oral Invest 22(3):1273–1283
24. Gonzalez-Martin O, Veltri M, Moraguez O, Belser UC (2014) Quantitative three-dimensional methodology to assess volumetric and profilometric outcome of subepithelial connective tissue grafting at pontic sites: a prospective pilot study. Int J Periodontics Restorative Dent 34(Suppl 5):673–679
25. Sanz Martin I, Benic GI, Hammerle CH, Thoma DS (2016) Prospective randomized controlled clinical study comparing two dental implant types: volumetric soft tissue changes at 1 year of loading. Clin Oral Implants Res 27(4):406–411
26. Vignoletti F, Abrahamsson I (2012) Quality of reporting of experimental research in implant dentistry. Critical aspects in design, outcome assessment and model validation. J Clin Periodontol 39(Suppl. 12):6–27
27. Schwarz F, Herten M, Ferrari D, Wieland M, Schmitz L, Engelhardt E (2007) Guided bone regeneration at dehiscence-type defects using biphasic hydroxyapatite + beta tricalcium phosphate (Bone Ceramic) or a collagen-coated natural bone mineral (BioOss Collagen): an immunohistochemical study in dogs. Int J Oral Maxillofac Surg 36(Suppl 12):1198–1206
28. Lee IK, Lim HC, Lee JS, Hong JY, Choi SH, Jung UW (2016) Layered approach with autogenous bone and bone substitute for ridge augmentation on implant dehiscence defects in dogs. Clin Oral Implants Res 27(Suppl 5):622–628
29. Artzi Z, Weinreb M, Givol N, Rohrer MD, Nemcovsky CE, Prasad HS, Tal H (2004) Biomaterial Resorption Rate and Healing Site Morphology of Inorganic Bovine Bone and β-Tricalcium Phosphate in the Canine: A 24-month Longitudinal Histologic Study and Morphometric Analysis. Int J Oral Maxillofac Implants 19(Suppl 3):357–368
30. Mordenfeld A, Hallman M, Johansson CB, Albrektsson T (2010) Histological and histomorphometrical analyses of biopsies harvested 11 years after maxillary sinus floor augmentation with deproteinized bovine and autogenous bone. Clin Oral Implants Res 21(Suppl 9):961–970
31. Jensen SS, Broggini N, Hjørting-Hansen E, Schenk R, Buser D (2006) Bone healing and graft resorption of autograft, anorganic bovine bone and β-tricalcium phosphate. A histologic and histomorphometric study in the mandibles of minipigs. Clin Oral Implants Res 17(Suppl 3):237–243
32. Sanz M, Ferrantino L, Vignoletti F, De Sanctis M, Berglundh T (2017) Guided bone regeneration of non-contained mandibular buccal bone defects using deproteinized bovine bone mineral and a collagen membrane: an experimental in vivo investigation. Clin Oral Implant Res 28(11):1466–1476
33. Cha JK, Joo MJ, Yoon S, Lee JS, Choi SH, Jung UW (2017) Sequential healing of only bone grafts using combining biomaterials with cross-linked collagen in dogs. Clin Oral Implants Res 28: 76–85
34. Schwarz F, Rothamel D, Herten M, Sager M, Becker J (2006) Angiogenesis pattern of native and cross-linked collagen membranes: an immunohistochemical study in the rat. Clin Oral Implants Res 17(Suppl 4):403–409
35. Friedman A, Gissel K, Soudan M, Kleber BM, Pitaru S, Dietrich T (2011) Randomized controlled trial on lateral augmentation using two collagen membranes: morphometric results on mineralized tissue compound. J Clin Periodontol 38(7):677–685
36. Bornstein MM, Bosshardt D, Buser D (2007) Effect of two different bioabsorbable collagen membranes on guided bone regeneration: a comparative histomorphometric study in the dog mandible. J Periodontol 78(Suppl 10):1943–1953
37. Becker J, Al-Nawas B, Klein MO, Schliephake H, Terheyden H, Schwarz F (2009) Use of a new cross-linked collagen membrane for the treatment of dehiscence-type defects at titanium implants: a prospective, randomized-controlled double-blinded clinical multicenter study. Clin Oral Implants Res 20(Suppl 7):742–749
38. Jemt T, Lekholm U (2003) Measurements of buccal tissue volumes at single-implant restorations after local bone grafting in maxillas: a 3-year clinical prospective study case series. Clin Implant Dent Relat Res 5(Suppl 2):63–70

39. Jemt T, Lekholm U (2005) Single implants and buccal bone grafts in the anterior maxilla: measurements of buccal csrystal contours in a 6 year prospective clinical study. Clin Implant Dent Relat Res 7(3):127–135
40. Henriksson K, Jemt T (2004) Measurements of soft tissue volume in association with single implant restoration: a 1 year comparative study after abutment connection surgery. Clin Implant Dent Relat Res 6(4):181–189
41. Schneider D, Gründer U, Ender A, Hammerle CH, Jung RE (2011) Volume gain and stability of peri-implant tissue following bone and soft tissue augmentation: 1 year results from a prospective cohort study. Clin Oral Implant Res 22(1):28–37

***Artículo 2**: Hard and soft tissue changes after guided bone regeneration using two different barrier membranes. An experimental in vivo investigation. Di Raimondo R, Sanz-Esporrin J, Sanz-Martin I, Pla R, Luengo F, Vignoletti F, Nuñez J, Sanz M. Clin Oral Investig. (2020) doi: 10.1007/s00784-020-03537-5*

Objetivo. Evaluar los cambios volumétricos y del contorno vestibular de los tejidos blandos y duros tras regeneración ósea guiada en defectos de dehiscencias peri-implantarias, con dos membranas barrera diferentes.

Material y métodos. Tras la hemisección y las extracciones de los dientes (M1M, Pm4M, Pm3D y Pm2), se crearon tres defectos óseos en caja en cada hemi-mandíbula de los perros. Ocho semanas después, se procedió primero a la colocación de dos implantes en cada defecto, y luego se realizó la regeneración ósea horizontal en las dehiscencias vestibulares creadas previamente. Los defectos se aleatorizaron y se asignaron cada uno a un grupo diferente. i) Como test se usó la combinación de un xenoinjerto bovino (DBBM) cubierto por una membrana sintética a base de ácido poliláctico; ii) como control positivo se usó la combinación del mismo xenoinjerto bovino (DBBM) cubierto por una membrana de colágeno nativo porcino; iii) en el control negativo se colocó solamente la membrana del grupo test y sin ningún sustituto óseo *(Imagen Suplementaria 3)*. Estos procedimientos (extracciones, creación de defectos óseos, colocación de implantes y ROG) se repitieron en las hemi-mandíbulas contralaterales después de 8 semanas, y el sacrificio tuvo lugar después de otras 4 semanas, lo que se traduce en dos periodos de curación diferentes (4 y 12 semanas).

Resultados. A pesar de que en los tres grupos se observó un aumento significativo del volumen y del contorno de ambos tejidos blandos y duros, los resultados mostraron una superioridad del grupo test y del control positivo en comparación con el control negativo, aunque las diferencias entre los grupos no fueron significativas.

Conclusiones. La ROG con un xenoinjerto combinado con una membrana sintética o de colágeno nativo permite obtener resultados similares, en términos de cambios volumétricos y del contorno de ambos tejidos blandos y duros, en comparación con la sola membrana.

Clinical Oral Investigations
https://doi.org/10.1007/s00784-020-03537-5

ORIGINAL ARTICLE

Hard and soft tissue changes after guided bone regeneration using two different barrier membranes: an experimental in vivo investigation

Riccardo Di Raimondo[1] · Javier Sanz-Esporrín[1,2] · Ignacio Sanz-Martin[1] · Rafael Plá[1] · Fernando Luengo[1] · Fabio Vignoletti[1,2] · Javier Nuñez[1] · Mariano Sanz[1,2]

Received: 1 April 2020 / Accepted: 18 August 2020

Abstract

Objective To assess the contour and volumetric changes of hard and soft tissues after guided bone regeneration (GBR) using two types of barrier membranes together with a xenogeneic bone substitute in dehiscence-type defects around dental implants.

Material and methods In 8 Beagle dogs, after tooth extraction, two-wall chronified bone defects were developed. Then, implants were placed with a buccal dehiscence defect that was treated with GBR using randomly: (i) deproteinized bovine bone mineral (DBBM) covered by a synthetic polylactic membrane (test group), (ii) DBBM plus a porcine natural collagen membrane (positive control) and (iii) defect only covered by the synthetic membrane (negative control group). Outcomes were evaluated at 4 and 12 weeks. Micro-CT was used to evaluate the hard tissue volumetric changes and STL files from digitized cast models were used to measure the soft tissues contour linear changes.

Results Test and positive control groups were superior in terms of volume gain and contour changes when compared with the negative control. Soft tissue changes showed at 4 weeks statistically significant superiority for test and positive control groups compared with negative control. After 12 weeks, the results were superior for test and positive control groups but not statistically significant, although, with a lesser magnitude, the negative control group exhibited gains in both, soft and hard tissues.

Conclusions Both types of membranes (collagen and synthetic) attained similar outcomes, in terms of hard tissue volume gain and soft tissue contours when used in combination with DBBM

Clinical relevance Synthetic membranes were a valid alternative to the "gold standard" natural collagen membrane for treating dehiscence-type defects around dental implants when used with a xenogeneic bone substitute scaffold

Keywords Guided bone regeneration · Synthetic barrier membrane · Collagen membrane · Prophilometric and volumetric changes · Dental implant · Animal model

MeSH terms Pre-clinical in vivo investigation · Alveolar bone loss · Dental implants · Guided bone regeneration · Barrier membranes

Introduction

Tooth loss is usually the consequence of the extraction of teeth with advanced stages periodontitis or due to trauma, root fractures or endodontic failures [1]. These situations may result in hard and soft tissue deficiencies [2, 3] and its prosthetic rehabilitation, usually by placement of dental implants, although demonstrating high long-term survival rates [4], not always restore the ideal tissue profile [5, 6]. Moreover, there are frequent situations in which the available bone volume is not sufficient for the placement of implants [7] or when placed, these implant-supported restorations cannot restore the

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00784-020-03537-5) contains supplementary material, which is available to authorized users.

✉ Mariano Sanz
marsan@ucm.es

[1] Postgraduate Periodontology Clinic, Faculty of Odontology, University Complutense of Madrid, Madrid, Spain

[2] ETEP (Etiology and Therapy of Periodontal and Periimplant Diseases) Research Group, University Complutense of Madrid, Madrid, Spain

Published online: 26 August 2020

appropriate hard and soft tissue contours and hence an acceptable aesthetic outcome [5, 6]

To achieve the appropriate alveolar bone crest for implant placement or for implant healing, lateral bone augmentation mainly through "guided bone regeneration" using bone replacement grafts and a barrier membrane has shown predictable outcomes, when carried out prior or in conjunction with implant placement [7, 8]. Bone replacement grafts are used to maintain the space to be regenerated and to act as a scaffold for bone conduction and eventually for bone regeneration [9–11]. Among the different bone replacement grafts, bovine xenografts have reported predictable outcomes in lateral bone augmentation, both prior and in conjunction with implant placement [11, 12]. The principles of GBR are not only based on the use of a bone replacement graft but also on its coverage with a barrier membrane that allows the homing effect of cells with osteogenic capacity from the bone defect walls able to colonize the bone replacement graft and eventually to replace this volume with newly formed bone, by excluding the soft tissue in growth in the defect [13, 14]. Non-resorbable membranes have shown predictable outcomes in promoting bone regeneration, but have also demonstrated more early exposure, with more bacterial contamination and greater incidence of would dehiscence, which jeopardizes the regenerative outcomes [15, 16]. For these reasons, bioabsorbable membranes have become the state of the art in GBR procedures. The most widely used are those made of native porcine collagen, which have shown in both small and large animal models, good barrier capability with concomitant promotion of cell migration and angiogenesis [9, 17, 18]. They have, however, the disadvantage of their low rigidity, which results in its collapse into the defect and thus prevents its capacity for space maintenance [7]. Furthermore, native collagen membranes have demonstrated to markedly reduce their thickness between 2 and 4 weeks after implantation and, depending on the animal model studied, being completely bioabsorbed between 4 and 12 weeks, what may limit their barrier properties, mainly in large bone defects [17–19]. Due to this, a new generation of synthetic biodegradable membranes has been developed aimed to improve its physical properties and their rate of biodegradability. They are made out of aliphatic polyesters: poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(epsilon-caprolactone) (PCL), poly(hydroxyl Valerie acid), poly (hydroxyl butyric acid) and their copolymers [14, 20]. Among these, GUIDOR® matrix barrier is manufactured with a double layer of polylactic acid blended with acetyltributylcitrate. It has shown high flexibility that allows good adaptation to the anatomy of different bone defects, as well as stiffness for space maintenance. Its macro-design includes the presence of an external layer with large pores aimed to promote soft tissue integration and an inner layer composed of small pores that prevents soft tissue ingrowth to the defect and promotes blood clot stabilization and new bone formation. Although most of the scientific documentation of this synthetic membrane has been reported when used in periodontal regeneration indications [21, 22], it has also been tested as the barrier membrane in GBR procedures to treat peri-implant dehiscence defects [23, 24].

To evaluate the mechanisms of action and tissue changes associated with the use of different bone replacement grafts and barrier membranes in GBR procedures, histological outcomes in pre-clinical in vivo investigations are currently the state of the art experimental design. However, these techniques only provide information related to a chosen section, usually 20–50 microns in thickness, what may prevent from assessing relevant information, mainly in terms of volume regeneration and contour reconstruction. With the advent of modern digital technologies, it is currently possible to obtain information of volumetric and contour linear changes of both hard and soft tissues by superimposing virtual stereolithographic (STL) models [25–29]. Furthermore, the use of high-resolution micro-Ct allows the quantification of hard tissue volumes and the 3-D bone micro-architecture. Recently, pre-clinical studies have shown the efficacy of micro-CT to study bone regenerative outcomes in the treatment of critical osseous defects [30, 31].

It was therefore the objective of this in vivo pre-clinical investigation to assess the volumetric changes in hard tissues and the soft tissue contour linear changes by micro-CT and STL superimposition analysis, after a simultaneous GBR lateral bone augmentation procedure in the treatment of peri-implant bone dehiscence defects, comparing a synthetic bioabsorbable membranes with the most frequently reported native collagen membrane.

Material and methods

Study design

This pre-clinical in vivo investigation was designed as a prospective randomized trial comparing three bone regenerative interventions following the ARRIVE guidelines [32]. The protocol of the study had been previously approved by the Regional Ethics Committee for Animal Research (EXP-20170327). The experimental phase was carried out at the Experimental Surgical Department of the Minimally Invasive Surgery Centre, Cáceres, Spain. The micro-CT data acquisition and three dimensional analysis was performed at the Biomaterials, Biomechanics & Tissue Engineering group at the Universidad Politécnica de Cataluña, Spain, and the volumetric and prophylometric analysis were carried out by the ETEP Research Group at the Complutense University, Madrid, Spain, and the Faculty of Veterinary, at the University of Santiago de Compostela in Lugo, Spain

Study population

Eight young female Beagle dogs (12–24 months), weighted between 10 and 15 kg, were used for the study. All animals were observed 2 weeks prior to the experiment phase to assess their general health status and none presented any systemic condition that could interact with the surgeries or the healing processes. Each animal was identified by a subcutaneous chip that was maintained during the study and all was monitored daily by an experienced veterinarian. Animals were maintained in individual kennels in a light/darkness light/darkness cycle of 12:12 with a temperature of 21–22°. Alimentation was based on hard animal food specific for this species and with free access to water.

Surgical interventions

Surgical interventions were performed in a period of 20 weeks. In all the surgical interventions, animals were sedated with propofol (2 mg/kg/i.v., Propovet, Abbott Laboratories, Kent, UK) and placed under general anaesthesia with 2.5–4% of isoflurane (Isoba-vet, Schering-Plough, Madrid Spain). Local anaesthesia with vasoconstriction (lidocaine 2% with epinephrine 1:100,000) (2% Xylocaine Dental, Dentsply, York, PA, USA) was also infiltrated to control bleeding during the surgery.

In the first surgical intervention extractions of the mesial roots of M (molar)1 and PM (premolar)4, distal roots of PM 3 and second PM were performed in one side of the mandible (Fig. 1a, b). The remaining hemisected roots were treated with calcium hydroxide (Dycal, Dentsply, York, USA) to avoid any possible pulp affectation. Immediately after the extractions, three standardized box-shaped bone defects (10 mm apico-coronally, 10 mm mesio-distally and 5 mm bucco-lingually) were created in each hemimandible using bone burs with profuse irrigation (Fig. 1c). Finally, flaps were sutured with absorbable sutures (Vicryl® 4.0, Johnson e Johnson, ST-Stevens-Woluwe, Belgium).

In the second surgical intervention 8 weeks after, the same surgical procedures were carried out on the contralateral side of the mandible and in the previously treated hemimandible, GBR regenerative interventions were performed to treat the chronified defects (Fig. 1d). After raising a full-thickness mucoperiosteal flap two dental implants (Dentium® NR; 2.5 mm in diameter and 7 or 9 mm long, Suwon, Korea) were placed on each chronified defect, leaving a similar buccal dehiscence in all the implants, which was measured intra-surgically with a periodontal probe (Hu Friedy, Chicago, IL, USA) (Fig. 2a). After implant placement, three different regenerative procedures were randomly assigned and allocated to the three defects using a randomized block distribution. All regenerative interventions were performed by two experienced investigators (FV, JN) in pre-clinical experimental models, assuring that all defects were filled evenly, levelled with the buccal mandibular bone contour.

- In the test group, deproteinized bovine bone mineral (DBBM) (BioOss® Geistlich, Wolhusen, Switzerland) and a synthetic polylactic membrane (GUIDOR®, Sunstar, Switzerland) were utilized.
- In the positive control group, DBBM (BioOss® Geistlich, Wolhusen, Switzerland) and a natural porcine collagen membrane (BioGide® Geistlich, Wolhusen, Switzerland) were utilized.
- In the negative control group, only a synthetic polylactic membrane (GUIDOR®, Sunstar, Switzerland) with no bone substitute was utilized.

The membranes were stabilized with titanium pins in the buccal aspect of the crest and the flap was closed aiming for tension-free primary intention after performing periosteal releasing incisions (Fig. 2b, c).

In the third surgical intervention, the same regenerative procedures were performed on the contralateral hemimandible where defects were chronified for 8 weeks. While these defects represented the short healing period (4 weeks), the sites operated in the second surgery were left to heal for a long healing period (12 weeks).

Four weeks after the third surgical intervention, dogs were sacrificed using a lethal dose of sodium pentothal (40–60 mg/kg/i.v., Dolethal, Vetoquinol, France). Dissections of the intervened areas were performed for micro-CT analysis.

Volumetric hard tissue analysis: micro-CT image acquisition and data analysis

The obtained jaw specimens were segmented to isolate each treated defect and prepared for micro-CT scanning, image acquisition (DICOM) and processing. Specimens were analysed with a Skyscan 1272 (BRUKER) Micro-Ct Scan with the following configuration (resolution 20 μm, 0.2° step, 3 frame averaging, 360° scanning, filter Al 0.5 + Cu 0.038, image size 1008 × 672 px, scanning time 2 h). After image acquisition, data processing and 3D reconstruction were done with NRecon software (BRUKER) and Data Viewer software. A pre-defined volume of interest (VOI) was set in each sample, always located buccally to the implants and covering the area of both implants. With a pre-set dimension of 10 × 6 × 8 mm (defect regeneration volume) (Fig. 3a). The achieved mineralized tissue volume was quantified in each sample with the CTAn software (BRUKER) that uses thresholds of greyscale (Fig. 3b, c and d). In the same sections, the proportion of bone volume/total volume (BV/TV) was quantified at both healing periods. Moreover, horizontal and vertical linear measurements were assessed in micro-CT bucco-lingual

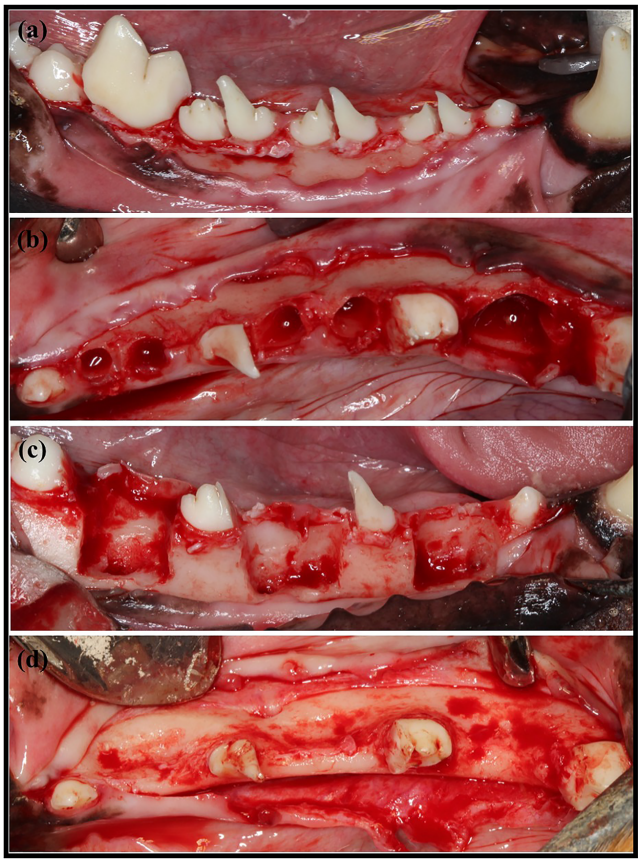


Fig. 1 Detail of the sequence in the experimental model for defect creation and chronification. **a**) Hemisection of P2, P3, P4 and M1 prior to tooth extraction. **b** Extractions of P2, distal root of P3, mesial root of P4 and mesial root of M1. **c** Experimental bone defect creation in rectangular boxes of 10 mm apicocoronally, 10 mm mesio-distally and 5 mm bucco-lingually. **d** Final two-wall defect morphology after 8 weeks of healing

sections (Fig. 3e). While one vertical measurement was registered for each section and corresponded to the distance between the implant shoulder and the first mineralized tissue to implant contact, three horizontal measurements, at three different points below the implant shoulder (0 mm, 2 mm and 4 mm), evaluated the buccal width of mineralized tissue. These measurements were performed using an image analysis software (ImageJ, Bethesda, Maryland, USA) and expressed in millimetres (mm).

Stereolithography image acquisition, matching and soft tissue contour linear change measurements

For image acquisition, matching and contour analysis, we used the same protocol recently published by our research group in a similar pre-clinical in vivo investigation [29]. In brief, individualized trays were fabricated for each dog (Fig. 4a) and before each surgical intervention, silicon impressions of both mandibles were taken using a light/heavy putty (Elite HD +, Zhermack spa, RO, Italy) (Fig. 4b, c). Cast models were then poured with stone (Fujirock type 4, GC. Corp, Tokyo, Japan) (Fig. 4d) and labelled appropriately to clearly distinguish the different healing periods. Obtained models were then optically scanned using a desktop 3D scanner (Zfx Evolution Scanner, Zimmer Dental, Bolzano, Italy) and the obtained stereolithographic (STL) files were measured and compared for each hemimandible (Fig. 4e). Baseline measurements corresponded to hemimandibles after healing of the surgically created defects and prior to the GBR procedure. Subsequent impressions and models were taken immediately before the sacrifice of the animals, then producing STL data for two healing periods, at 4 and 12 weeks after the GBR procedures.

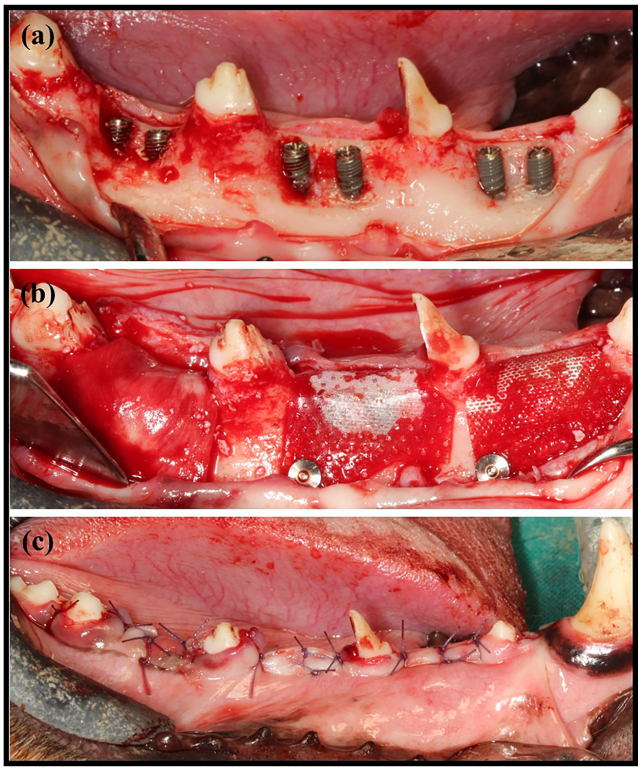


Fig. 2 Picture of GBR procedure. a Implant placement with vestibular dehiscences in all the three defects in the same hemimandible. b Defects filled with the three different combination of biomaterials. c. Primary close of the flap with absorbable sutures

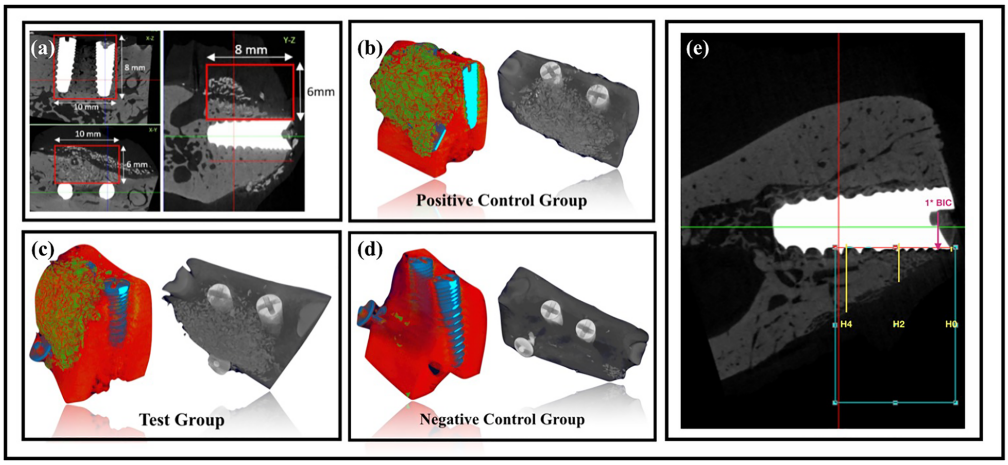


Fig. 3 Picture of the hard tissue analysis. a Area of interest used for volumetric analysis. b Section of a positive control group. c Section a the test group. d Section of a negative control group. e Horizontal linear micro-Ct radiographic measurements at three different heights below the implant shoulder (0 mm, 2 mm, 4 mm) and one vertical measurement represented the first hard tissue contact

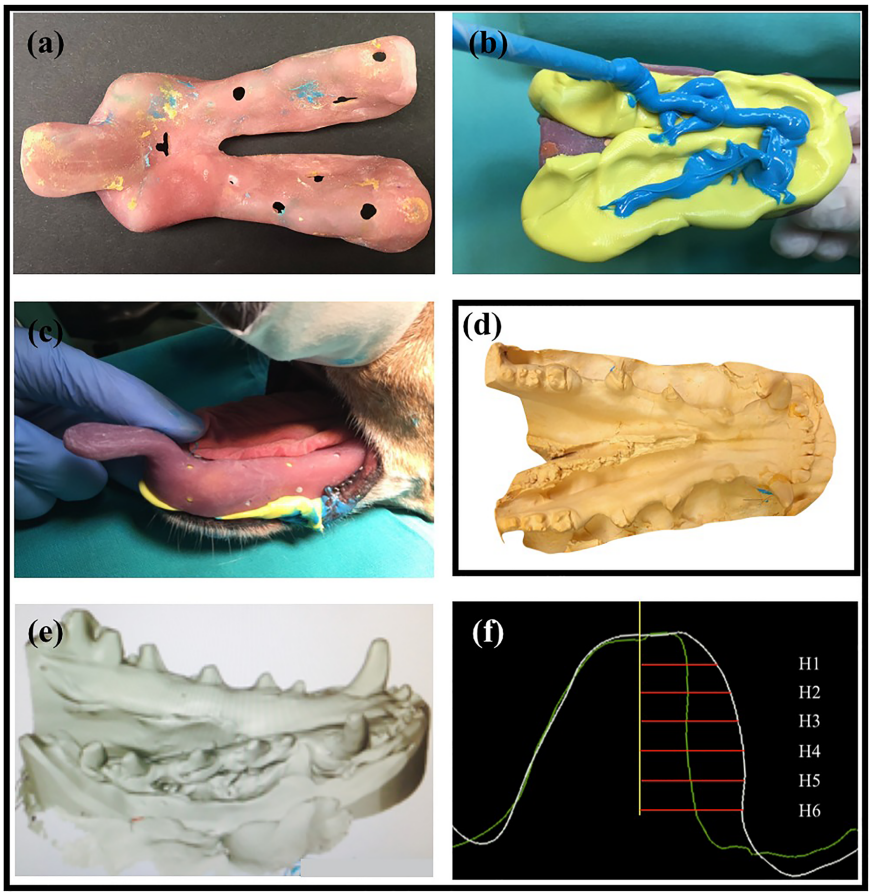


Fig. 4 Picture of the soft tissue analysis. a Individual tray of a dog used for the entire investigation. b Placement of both light and heavy putty silicon inside the individual tray. c Impression taking of the mandible of a dog. d Cast model of the mandible of a dog poured with dental stone. e Obtention of the Stl file following the scanner of the cast model with an optical scanner. f Stl files and linear measurement between pre-GBR and post-GBR files at six different heights from the rim of the crest; the green line represents pre-GBR contour, while the white line represents post-GBR contour. Red lines show the measurements performed inside the defect

Obtained STL files were imported to an image analysis software (SMOP, Swissmeda Software, Swissmeda AG, Zurich, Switzerland) allowing for superimposition (matching) using four or five pairs of points of fixed references. The software then performed a "rough fit" and then, additional points were selected to perform a "fine fit" superimposition of the two models. Minor discrepancies were improved manually by adjusting the position of the models.

Once the models were matched, a longitudinal buccolingual section was selected in the middle part of each defect, obtaining a cross-sectional section that divided each defect mesio-distally into two equal parts. Then, a vertical line coinciding with the axis of the tooth prior to its extraction was drawn in the middle of each cross-sectional image. A screenshot of each of these transversal sections was finally taken and uploaded into another analysis software (OLYMPUS® cellSens Dimension Desktop 1.14) which was used to measure the contour linear changes.

The analysis of the contour changes was done using perpendicular lines drawn at 1, 2, 3, 4, 5 and 6 mm from the most coronal part of the baseline crest. The length of each line represented the width of the buccal crest at both baseline and post-GBR periods. To register the contour changes of the buccal crest at these six different heights, the differences

Table 1 Micro-Ct volumetric data of hard tissue at both healing periods (4–12 weeks). All measurements are expressed in cubic millimetres

Healing	Group	Mean (SD)	Mean differences	IC 95%		*p*
				Inf. Lim	Sup. Lim	
4 weeks	*T*	88.40 (46.02)	16.58*	− 42.65	75.81	1.00
	+ *C*	71.81 (59.00)	32.34†	− 26.88	91.58	0.51
	− *C*	39.47 (24.94)	48.93§	− 10.30	108.16	0.13
12 weeks	*T*	45.54 (25.24)	− 10.35*	− 50.85	30.41	1.00
	+ *C*	55.76 (40.65)	20.57†	− 20.05	61.21	0.606
	− *C*	35.19 (25.25)	10.35§	− 30.28	50.99	1.00
Total	*T*	66.97 (42.13)	3.18*	− 32.11	38.47	1.00
	+ *C*	63.79 (49.64)	26.46†	− 8.82	61.75	0.206
	− *C*	37.33 (24.35)	29.64§	− 5.64	64.93	0.127

*Test vs control +

†Control + vs control –

§Test vs control − ; *SD*, standard deviation; *IC 95%*, confidence interval 95%

between the buccal width at the follow-up visit minus the buccal width at the baseline visit were calculated, thus obtaining the contour linear change in millimetres (mm) (Fig. 4f). These values were then stratified as coronal (1 mm below the most coronal aspect of the ridge), intermediate (3 mm) and apical (5 mm) levels in order to better understand the behaviour of the biomaterials inside the defect.

Data analysis

Randomization of the interventions was performed using a computer-generated list that considered the side and position in the jaw (mesial, central or distal). Linear contour changes were calculated for each period (baseline 4 weeks/baseline 12 weeks) and expressed as means, standard deviation (SD), confidence intervals and frequency distributions. Similar descriptive data was generated for the hard tissue volume changes at 4 and 12 weeks of healing.

Shapiro-Wilk normality tests were performed to assess the data distribution. A general linear model was used to assess for multiple comparisons between absolute measurements of the primary outcome variable ("contour change"), considering the treatment group and healing time. The same tests were performed for the secondary outcome variable "hard tissue volume". ANOVA tests were used for the intergroup comparisons, as well as for the differences depending on the height of the linear contour measurement (crestal, intermediate and apical measurements at 1, 3 and 5 mm from the rim of the crest) and the position of the defect (mesial, central or distal). Bonferroni corrections were performed for multiple

Table 2 Micro-Ct volumetric data of BV/TV at both healing periods (4–12 weeks). All measurements are expressed in cubic millimetres

Healing	Group	Mean (SD)	Mean differences	IC 95%		*p*
				Inf. Lim	Sup. Lim	
4 weeks	*T*	24.15 (14.06)	0.92*	− 41.97	43.83	1.000
	+ *C*	23.22 (16.16)	− 1.86†	− 44.77	41.03	1.000
	− *C*	25.09 (36.44)	− 0.93§	− 43.84	41.96	1.000
12 weeks	*T*	13.43 (7.75)	− 11.67*	− 36.11	12.75	0.626
	+ *C*	25.11 (20.52)	13.10†	− 11.32	37.54	0.485
	− *C*	12.01 (9.92)	1.42§	− 23.00	25.86	1.000
Total	*T*	18.79 (12.10)	− 5.37*	− 27.55	16.80	1.000
	+ *C*	24.17 (17.44)	5.62†	− 16.55	27.79	1.000
	− *C*	18.55 (26.10)	0.24§	− 21.93	22.42	1.000

*Test vs control +

†Control + vs control –

§Test vs control –; *SD*, standard deviation; *IC 95%*, confidence interval 95%

Table 3 Linear micro-Ct radiographic measurements for both healing periods (4–12 weeks). All measurements are expressed in millimetres

Healing	Measurement	Mm	Group	Mean (SD)	Mean differences	IC 95%		*p*
						Inf. Lim.	Sup. Lim.	
4 weeks	Horizontal	0	*T*	0.22 (0.43)	− 0.39 *	− 1.36	0.57	0.896
			C +	0.61 (1.18)	0.53 †	− 0.43	1.49	0.503
			C −	0.08 (0.24)	0.13 §	− 0.83	1.10	1.000
		2	*T*	2.29 (0.81)	0.51*	− 1.31	2.34	1.000
			C +	1.77 (1.93)	0.48†	− 1.34	2.31	1.000
			C −	1.29 (1.24)	0.99§	− 0.83	2.82	0.517
		4	*T*	3.40 (1.70)	0.62*	− 1.48	2.73	1.000
			C +	2.77 (1.85)	0.81†	− 1.29	2.91	0.983
			C −	1.96 (1.23)	1.43§	− 0.66	3.54	0.271
	Vertical		*T*	0.76 (0.64)	− 0.88*	− 2.52	0.75	0.524
			C +	1.64 (1.46)	− 0.66†	− 2.31	0.97	0.906
			C −	2.31 (1.49)	− 1.55§	− 3.19	0.08	0.067
12 weeks	Horizontal	0	*T*	0	− 0.36*	− 1.03	0.29	0.494
			C +	0.36 (0.70)	0.09†	− 0.57	0.75	1.000
			C −	0.27 (0.53)	− 0.27§	− 0.93	0.39	0.890
		2	*T*	0.56 (0.87)	− 0.15*	− 1.55	1.24	1.000
			C +	0.72 (1.28)	0.13†	− 1.26	1.53	1.000
			C −	0.58 (1.03)	− 0.02§	− 1.42	1.37	1.000
		4	*T*	1.32 (0.65)	− 0.40*	− 1.85	1.04	1.000
			C +	1.72 (1.13)	0.01†	− 1.43	1.45	1.000
			C −	1.71 (1.42)	− 0.39§	− 1.84	1.05	1.000
	Vertical		*T*	2.07 (1.40)	0.19*	− 1.65	2.04	1.000
			C +	1.88 (1.35)	− 0.50†	− 2.35	1.34	1.000
			C −	2.39 (1.49)	− 0.31§	− 2.16	1.53	1.000
Total	Horizontal	0	*T*	0.11 (0.31)	− 0.38*	− 0.93	0.16	0.275
			C +	0.49 (0.95)	0.31†	− 0.23	0.86	0.495
			C −	0.18 (0.41)	− 0.06§	− 0.62	0.48	1.000
		2	*T*	1.42 (1.20)	0.17*	− 1.02	1.38	1.000
			C +	1.25 (1.67)	0.30†	− 0.89	1.51	1.000
			C −	0.94 (1.16)	0.48§	− 0.71	1.69	0.961
		4	*T*	2.36 (1.64)	0.11*	− 1.22	1.44	1.000
			C +	2.25 (1.58)	0.41†	− 0.92	1.74	1.000
			C −	1.84 (1.29)	0.52§	− 0.80	1.85	1.000
	Vertical		*T*	1.42 (1.25)	− 0.34*	− 1.54	0.84	1.000
			C +	1.76 (1.36)	− 0.58†	− 1.78	0.60	0.686
			C −	2.35 (1.44)	− 0.93§	− 2.12	0.26	0.174

SD, standard deviation; *IC 95%*, confidence interval 95%

*Test vs control +

†Control + vs control –

§Test vs control –

comparisons. The alpha error was set at 0.05 and all tests were performed with the statistical software package (IBM SPSS Statistics® V20 JM.Domenech).

Results

Healing after the surgical interventions occurred uneventfully and all 8 dogs healed appropriately. Forty-eight defects were obtained for micro-CT analysis (16 test, 16 positive and 16 negative control groups), 24 for each healing period (4 and 12 weeks). For evaluation of the soft tissue changes, 45 defects were included since 3 digitized cast models belonging to the 12 weeks of healing, 1 from the test group and 2 from the positive control group were unreadable.

Therefore, for the digitized model analysis, 45 were selected, 24 corresponded to a 4-week healing period (8 test group, 8 positive control and 8 negative control groups) and 21- to 12-week healing period (7 test group, 6 positive control and 8 negative control groups).

Hard tissue micro-CT volumetric analysis

The intergroup comparisons at both healing periods (4–12 weeks) are depicted in Table 1. When all sample were analysed (T4 + T12), mean hard tissue volume was superior for the test group, followed by positive control and then the

negative control group: 66.97 mm3 (IC 95% − 32.11; 38.47), 63.79 mm^3 (IC 95% − 8.82; 61.75) and 37.33 mm3 (IC 95% − 5.64; 64.93) respectively, although these differences did not reach statistical significance ($p = 0.068$).

At the early healing time (4 weeks), the test group obtained more hard tissue volume compared with both positive and negative control groups (16.58 mm^3 more when compared with the positive control (IC 95% = − 42.65; 75.81) and 48.93 mm^3 more when compared with the negative control (IC 95% = − 10.30; 108.16). These differences, however, were not statistically significant ($p = 0.116$). At 12 weeks of healing, the positive control group still achieved higher hard tissue volumes when compared with both the test group (10.35 mm3 (IC 95% = -30.41: 50.85) and negative control groups (20.57 mm3 (IC 95% = -20.05 : 61.21)).

When comparing the volumetric changes between 4 and 12 weeks, more volume augmentation occurred during the first 4 weeks, irrespective of the groups, although the use of a scaffold (test and positive control groups) resulted in almost double hard tissue volume when compared with the negative control group. In the test group, the volumetric changes at the early and late healing times (4–12 weeks) were 88.40 mm^3 vs 45.54 mm^3, in the positive control group 71.81 mm^3 vs 55.76 mm^3 and in the negative control group 39.47 mm^3 vs 35.19 mm^3.

The ratios of BV/TV at both healing periods (4–12 weeks) are depicted in Table 2. Analysing all samples (T4 + T12), the test and negative control achieved similar ratios (18.79 mm^3 and 18.55 mm^3, respectively). The positive control, however, was slightly superior (24.17 mm^3), although these differences were not statistically significant ($p = 1.000$).

At the early healing time (4 weeks), BV/TV was similar between groups, with a slight superiority of negative control group, followed by the test group and then the positive control group (25.09 mm^3, 24.15 mm^3 and 23.22 mm^3, respectively). No statistically significant differences were found between groups.

At 12 weeks, the BV/TV ratio in positive control sites was slightly superior compared with 4 weeks (23.22 mm^3). In the test and negative control groups, the rations diminished with time (13.43 mm^3 and 12.01 mm^3, respectively). But, differences were not statistically significant.

Linear micro-CT measurements

The horizontal and vertical linear measurements of hard tissues are depicted in Table 3. The width of the buccal hard tissues was higher for all groups after 4 weeks of healing compared with 12 weeks of healing, irrespective of the different height levels (0, 2, 4 mm). When data from both time periods were analysed together (T4 and T12), a superiority of buccal hard tissue width in the test group was observed at the 2 and 4 mm height levels, followed by the positive and negative control groups, respectively. No statistically significant differences were found in all three heights when the study groups were compared.

In early healing (4 weeks), the test group attained higher horizontal contour at 2 and 4 mm, while the positive control group was superior at the level of the implant shoulder. At

Table 4 Prophilometric and mean linear changes. Intergroup comparisons at both healing periods (4–12 weeks). All measurements are expressed in millimetres

Healing	Mm	Δ *T*/*C*+ (mm)	IC 95%		*p*	Δ *T*/*C*− (mm)	IC 95%		*p*	Δ *C*+/*C*− (mm)	IC 95%		*p*
			Inf. Lim.	Sup. Lim.			Inf. Lim.	Sup. Lim.			Inf. Lim.	Sup. Lim.	
4 weeks	1	0.15	− 0.65	0.96	1.00	0.45	− 0.35	1.26	0.47	0.29	− 0.50	1.10	1.00
	2	0.22	− 0.66	1.11	1.00	0.92	0.04	1.81	*0.03	0.70	− 0.18	1.59	0.15
	3	0.18	− 0.83	1.21	1.00	1.26	0.20	2.32	*0.01	1.07	0.01	2.13	*0.04
	4	0.06	− 1.05	1.17	1.00	1.33	0.18	2.49	*0.02	1.27	0.12	2.43	*0.02
	5	− 0.39	− 1.60	0.81	1.00	1.28	0.07	2.49	*0.03	1.67	0.42	2.92	*0.007
	6	− 0.79	− 2.30	0.70	0.51	1.47	0.13	2.81	*0.02	2.27	0.71	3.82	*0.04
12 weeks	1	0.36	− 0.98	1.72	1.00	0.66	− 0.59	1.92	0.53	0.29	− 1.01	1.61	1.00
	2	0.47	− 1.12	2.06	1.00	0.91	− 0.56	2.40	0.36	0.44	− 1.10	1.99	1.00
	3	0.51	− 1.11	2.14	1.00	1.42	− 0.09	2.93	0.07	0.90	− 0.68	2.48	0.45
	4	0.71	− 0.92	2.36	0.79	1.64	0.11	3.17	*0.03	0.92	− 0.67	2.52	0.43
	5	0.89	− 0.88	2.66	0.58	1.31	− 0.55	3.17	0.23	0.42	− 1.44	2.28	1.00
	6	1.13	− 0.80	3.06	0.38	0.97	− 1.08	3.02	0.62	− 0.163	− 2.21	1.88	1.00

T, test; *C*+, positive control; *C*−, negative control

*Statistically significant ($p < 0.05$); Δ: mean difference; *IC 95%*, confidence interval 95%

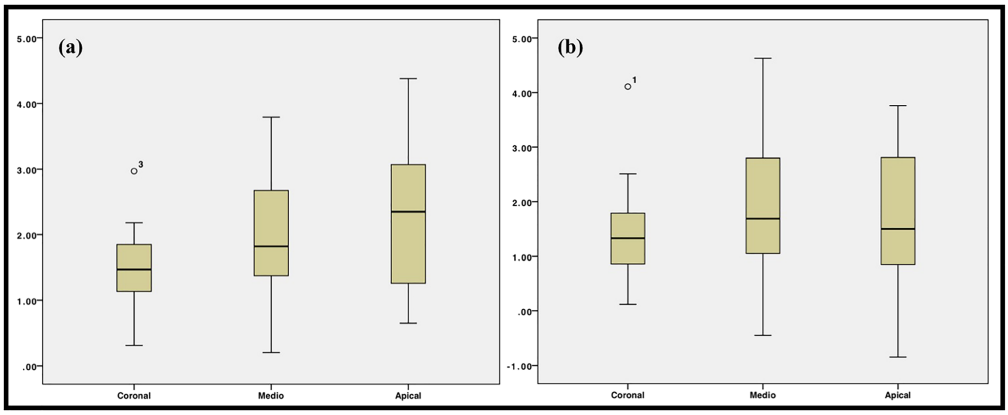


Fig. 5 Picture of the box plots representing the changes that occurred after both healing periods. a Box plot representing contour changes that took place after 4 weeks at three different heights of the crest, corresponded as coronal (1 mm), intermediate (3 mm) and apical (5 mm) levels. b Box plot representing contour changes that took place after 12 weeks at these three different levels of the crest (1, 3 and 5 mm)

12 weeks, the positive control group was superior in all heights (0, 2, 4 mm).

Vertical measurements showed that the first mineralized tissue to implant contact of the negative control group was always in a more apical position compared with test and positive control groups, irrespective of the healing times. After 4 weeks of healing, the first mineralized tissue to implant contact of the test group was in a more coronal position compared with positive control, whereas at 12 weeks, the positive control group was the one more coronally. All these differences, however, were not statistically significant among the treatment groups.

Soft tissue analysis

Baseline width measurements of the buccal crest were taken prior to the regenerative interventions at six different heights below the most coronal part of the rim of the crest (1, 2, 3, 4, 5 and 6 mm). In this context, the width of the crest for the short follow-up healing period (4 weeks) ranged between 3.05 mm (SD = 0.24) and 4.91 mm (SD = 1.08), while those at the long healing period (12 weeks) was between 2.88 mm (SD = 0.50) and 5.27 mm (SD = 1.16), at 1 and 6 mm from the rim of the crest, respectively. No statistically significant differences were found between the groups in all the heights studied (Supplementary Table 1).

Intragroup differences at both healing periods (4–12 weeks)

A statistically significant increase of the buccal contour was found for all three groups (test, positive and negative control groups) when comparing baseline and 4 and 12 weeks. Contour linear changes were superior between baseline and 4 weeks compared with the changes between baseline and 12 weeks (Supplementary Table 2).

Intergroup comparisons (4 and 12 weeks of healing) (Table 4)

After 4 weeks of healing, test and positive control groups demonstrated a higher increase in buccal contour compared with the negative control group at all the heights (1, 2, 3, 4, 5 and 6 mm). The comparison between the test group and the negative control group resulted in statistically significant higher contours at all heights of the crest except at 1 mm from the rim that in spite of being 0.45 mm (IC 95% = − 0.35; 1.26), this difference was not significant ($p = 0.47$). Similarly, the positive control group showed significant superiority in linear contour changes compared with the negative control group at all the heights, except at 1 and 2 mm from the rim, which were also superior but not significant ($p = 1.00$ and $p = 0.15$, respectively). The linear contour increase in the test group was similar when compared with the positive control group, being superior for the test only at 1, 2, 3 and 4 mm from the rim of the crest, although these differences were not statistically significant.

After 12 weeks, the soft tissue contours in the test group were superior compared with both positive and negative control groups at all the heights from the rim of the crest (1, 2, 3, 4, 5 and 6 mm), but differences were not statistically significant when compared with the positive control group. When compared with the negative control, however, at 4 mm, the difference was significant (1.64 mm; IC 95% = 0.11; 3.17) ($p = 0.03$). When the positive control group was compared with the negative control group, the soft tissue contours were also higher at all heights (1, 2, 3, 4 and 5 mm), except at the bottom of the defect (6 mm from the rim), where it was slightly

Table 5 Mean linear changes based on defect location and treatment group at both healing periods (4–12 weeks together)

Defect	Mm	Mean (SD) Test	Mean (SD) positive control	Mean (SD) negative control	Δ *T*/*C*+ (mm)	IC 95%		*p*	Δ *C*+/*C*− (mm)	IC 95%		*p*	Δ *T*/*C*− (mm)	IC 95%		*p*
						Inf. Lim	Sup. Lim			Inf. Lim	Sup. Lim			Inf. Lim	Sup. Lim	
A	1	1.55 (0.12)	2.02 (0.32)	0.96 (0.52)	−0.46	−2.09	1.16	1.00	1.06	−0.56	2.69	0.31	0.59	−1.18	2.37	1.00
	2	2.17 (0.21)	2.54 (0.52)	1.49 (0.14)	−0.37	−2.02	1.27	1.00	1.05	−0.59	2.69	0.33	0.67	−1.13	2.48	1.00
	3	2.86 (0.38)	3.02 (0.68)	1.64 (0.25)	−0.15	−2.18	1.87	1.00	1.38	−0.64	3.40	0.27	1.22	−0.99	3.44	0.50
	4	2.97 (0.71)	2.90 (1.22)	1.59 (0.67)	0.07	−2.38	2.53	1.00	1.31	−1.15	3.77	0.54	1.38	−1.31	4.08	0.59
	5	2.86 (0.53)	2.63 (1.53)	1.47 (0.87)	0.22	−2.42	2.88	1.00	1.16	−1.49	3.81	0.80	1.39	−1.51	4.29	0.68
	6	2.68 (0.22)	2.25 (2.02)	1.67 (1.13)	0.43	−2.55	3.41	1.00	0.58	−2.40	3.56	1.00	1.01	−2.25	4.27	1.00
B	1	0.98 (0.44)	1.27 (0.79)	1.21 (0.52)	−0.29	−1.48	0.90	1.00	0.61	−1.30	1.42	1.00	−0.22	−1.53	1.07	1.00
	2	1.14 (0.55)	1.52 (0.61)	1.04 (0.82)	−0.37	−1.58	0.83	1.00	0.48	−0.89	1.85	1.00	0.10	−1.21	1.42	1.00
	3	1.53 (1.03)	2.05 (0.57)	0.89 (0.63)	−0.51	−2.00	0.97	1.00	1.15	−0.53	2.85	0.27	0.63	−0.98	2.26	0.95
	4	1.83 (1.24)	2.38 (0.78)	0.92 (0.29)	−0.54	−2.35	1.26	1.00	1.46	−0.60	3.52	0.23	0.91	−1.05	2.88	0.72
	5	1.94 (1.18)	2.60 (1.11)	1.11 (0.47)	−0.66	−2.61	1.28	1.00	1.49	−0.72	3.71	0.28	0.83	−1.29	2.95	0.96
	6	2.02 (1.10)	2.67 (1.73)	1.33 (0.65)	−0.65	−2.84	1.54	1.00	1.34	−1.15	3.83	0.53	0.69	−1.69	3.07	1.00
C	1	1.93 (0.60)	1.60 (1.28)	0.96 (0.94)	0.33	−1.16	1.82	1.00	0.63	−0.86	2.12	0.85	0.96	−0.16	2.09	0.11
	2	2.32 (0.40)	2.16 (1.34)	0.74 (0.97)	0.16	−1.35	1.67	1.00	1.41	−0.98	2.92	0.07	1.57	0.43	2.71	*0.005
	3	2.60 (0.49)	2.25 (1.47)	0.90 (1.20)	0.35	−1.50	2.21	1.00	1.34	−0.51	3.20	0.22	1.70	0.29	3.10	*0.01
	4	2.76 (0.72)	2.10 (1.56)	1.01 (1.33)	0.66	−1.59	2.92	1.00	1.08	−1.17	3.34	0.67	1.75	0.04	3.45	*0.04
	5	3.02 (0.80)	1.75 (1.49)	1.03 (1.34)	1.27	−1.16	3.70	0.56	0.71	−1.72	3.14	1.00	1.98	0.14	3.82	*0.03
	6	3.14 (0.94)	1.29 (1.15)	1.21 (1.20)	1.85	−0.88	4.58	0.28	0.08	−2.65	2.81	1.00	1.93	−0.13	3.99	0.07

SD, standard deviation; *IC 95%*, confidence interval 95%; Δ: mean difference. A: mesial defect; B: central defect; C: distal defect

inferior of 0.16 (IC 95% = − 2.21; 1.88). These differences between positive and negative control groups were not statistically significant.

Contour changes depending on the apico-coronal level of the defects and defect location

For that purpose, the defects were divided as three parts to assess in which part of the defect more changes occurred, if it was at the most coronal part (1 mm from the rim), at the intermediate level (3 mm) or at apical level (5 mm) (Supplementary Table 3). After 4 weeks of healing, more change was found at the apical level followed by the intermediate and last the coronal level of the crest of 2.31 mm (1.11), 1.91 mm (0.93) and 1.44 mm (0.62), respectively (Fig. 5a). After 12 weeks of healing, however, more change was found at the intermediate level followed by the apical level and again, less contour was at the coronal level of the crest of 1.93 mm (1.22), 1.68 mm (1.19) and 1.43 mm (0.92), respectively (Fig. 5b)

Superiority in soft tissue contours for both test and positive control groups compared with the negative control group was attained irrespective of the defect location (Table 5), although the mesial defect was superior in terms of contour gain when compared with the central and distal defects. However, none of these differences were statistically significant (Supplementary Table 4).

Discussion

The results from this pre-clinical in vivo investigation have clearly demonstrated the reconstructive outcomes for both hard and soft tissues when applying the principles of GBR. At both healing periods, both hard tissue volume and soft tissue linear contour changes were superior in the test and positive control groups, when compared with the negative control group. Test and positive control groups used the same bone replacement graft (xenogeneic DBBM) and a synthetic polylactic-based membrane and a natural porcine collagen membrane, respectively, while the negative control group used the same synthetic membrane without the bone replacement graft. This study also demonstrated that the synthetic barrier membrane alone was able to significantly increase the hard tissue volume and soft tissue contours and in spite of a lesser magnitude of changes compared with the test and positive control groups, although these differences were not statistically significant, what shows the ability of this barrier membrane for space maintenance and tissue exclusion. Other pre-clinical studies, albeit using the rat model, have also shown significant bone augmentation measured by micro-CT after GBR using only a barrier membrane [31, 33].

In a recent pre-clinical study from our research group, similar results were shown for staged bone regeneration. Chronified defects with a similar defect size were filled with a bone graft composed of 90% of deproteinized bovine bone mineral with 10% collagen (DBBM-C), with an absorbable collagen membrane (NBCM) alone, or with the combination of both biomaterials [34]. Although the histological outcomes demonstrated that the degree of volume augmentation was significantly higher in both groups using a bone replacement graft, after 3 months in the middle part of the region of interest (ROI) membrane alone showed significantly higher new mineralized tissue when compared with either bone replacement graft alone or combined with a natural porcine collagen barrier. However, the main histological outcome of the combined effect of the bone replacement graft and the membrane was the presence of soft tissue biomaterial granule encapsulation when membranes were not used and a significantly higher new bone in contact with the biomaterial when the membrane was used [34]. Although no histological data was reported in the present study, similar findings in terms of encapsulation of biomaterial particles were also observed with the micro-Ct scans, irrespective of the type of membrane used. At 12 weeks, the BV/TV ratio was always superior for the groups combining bone replacement grafts and barrier membranes. Results evaluating changes in bone contour and volumetric changes of soft tissues from digitized cast models in a staged bone regeneration experimental model also reported the added value of combining a bone replacement graft and a barrier membrane [26].

Bioabsorbable barrier membranes of native porcine collagen have wide documentation in the scientific literature of their favourable handling properties and histological tissue integration, although due to their lack of cross-linking, they have limited rigidity and a rapid degradation rate (between 4 and 12 weeks) [9, 18, 19]. When compared in this investigation with a bioabsorbable synthetic polylactic-based membrane, the results in terms of mineralized hard tissue volume and soft tissue contour linear changes were similar, although the test group (synthetic membrane combined with the bone replacement graft) always attained superior outcomes, albeit non-statistically significant, mainly at the early healing phase (baseline 4 weeks). These outcomes could be explained by the higher degree of stiffness of the synthetic membrane, which may increase the space maintenance effect. Similar results were described in a similar pre-clinical model evaluating GBR histological outcomes comparing polylactic and collagen membranes at peri-implant dehiscence defects [24], where the early healing events provided higher volumetric changes in the polylactic membrane group. The use of this type of synthetic membrane combined with the basic fibroblast growth factor (bFGF) provided enhanced volume and density gains in new bone assessed with micro-Ct when compared with the use of the same membranes without the growth factor [23].

Pre-clinical studies comparing collagen and polylactic acid barrier membranes have shown that polylactic barrier membranes are degraded slower than native collagen membranes [24, 35]. This longer resorption rate is likely due to the double-layered structure of the bioresorbable polylactic acid barrier softened with a citric acid ester that facilitates soft tissue integration. This bio-absorption process lasts between 6 and 12 weeks through hydrolysis [21, 22], hence resulting in an acidic environment. In spite of the controversy on how this resulting low pH would affect new tissue formation, the use of these synthetic barrier membranes has shown significant regenerative outcomes in both pre-clinical and clinical studies [23, 24, 36].

Changes in hard tissue volume measured with micro-CT demonstrated increased mineralized tissue volume during the first 4 weeks of healing, together with a higher buccal contour of the soft tissues, although these increments decreased at 12 weeks. These results may be explained by the early degradation of the membranes and loss of the space maintenance effect and by the expected tissue contraction during later wound healing. The results in the horizontal linear measurements obtained with micro-CT were also similar since the increase in the buccal contour of mineralized tissue was higher at 4 weeks compared with 12 weeks of healings. These findings agree with data reported in a pre-clinical study in dogs where after GBR at dehiscence-type defects around implants, more hard tissue buccal contour was attained in early versus late healing times [37]. These findings could suggest that bone formation undergoes structural changes after the long healing periods but also less space maintenance of the membrane at the coronal aspect of the defects due to its collapse, with a displacement of the biomaterial apically, which directly affected bone formation, mainly in the most coronal aspect [37]. This fact could be reflected in the absence of complete regeneration of almost all of the dehiscence defects, at both healing periods. Complete regeneration was not observed neither in the test group nor in positive or negative control groups.

Moreover, an interesting finding was also observed with micro-Ct analysis, especially after 12 weeks. There are many cases in the micro-CT sections where the first mineralized tissue to implant contact was more apical in relation with the biomaterial particles situated in a more coronal position but not in contact with the implant. In these cases, the measurement of the first mineralized tissue to implant contact was more apical (generally around 2 mm below the implant shoulder), but the horizontal measurement in height H = 0 mm (implant shoulder) reveals the presence of biomaterial embedded in tissue, thus providing an enhanced horizontal contour and therefore volume gain (Supplementary Fig. 1).

In the present study, also soft tissue contour gains were not uniform along the buccal alveolar crest with higher gains at the mid and apical level and limited changes in the most coronal sites. Similar results have been previously reported in other pre-clinical experimental studies and have been explained by the effect of the soft tissue lateral pressure and the likely displacement of the biomaterial granules apically [29, 38].

The use of digitized cast models to generate STL files that can be compared over time, once the adequate superimposition of the models has been achieved, has clear advantages in the study of soft tissue changes and aesthetic outcomes, due to its reproducibility and attainment of measurable linear changes. This methodology has been used in both pre-clinical and clinical studies [25–29, 39]. However, the study of soft tissue contours does not provide information on the behaviour of the bone regenerative technologies used and hence, the use of micro-Ct for measuring the volumetric hard tissue changes allows to study these hard tissue changes but also to study the bone micro-architecture and the changes in bone density over time [23, 24, 40]. Micro-Ct analysis to assess the outcomes of GBR has been documented in several pre-clinical investigations, both in small and large animal models [23, 24, 31, 35, 37]. Recently, the combined analysis of DICOM files obtained from the micro-CT with STL files from digitized casts allowed the consecutive evaluation of both hard and soft tissue changes [41]. However, this software was not valid for this model since it was designed to evaluate the volume around implants after the connection of transmucosal abutments. Since the implants in this investigation were submerged this analysis was not possible. Other limitations in this investigation are related to the use of micro-Ct to evaluate bone regeneration since this technique assesses greyscale and it is not always possible to differentiate between mature native bone and the biomaterial used as a bone replacement graft. For this reason, we combined both mineralized tissues into one measurement (mineralized tissue).

Furthermore, although two implants were placed in each defect and the distance between them was always the same, it was not possible to perform the measurements of the soft tissue contour linear analysis at implant levels since they were submerged and we did not have any references point to determine the exact location of the implant. Due to this, the contour analysis was done at the centre of each defect, as being the most reproducible measurement site.

In light of the pre-clinical nature of this investigation and the referred limitations, the results reported in this study should be interpreted with caution since they may not translate directly to the clinical situation. Lastly, although micro-CT images provide good information on the bone volume and density, the lack of histological outcomes limits the evaluation of the possible tissue differences obtained under these two tested barrier membranes.

Taking into account these limitations, it was possible to conclude that GBR combining a xenogeneic bone replacement graft and a bioabsorbable membrane (test and positive

control groups) achieved superior outcomes in terms of hard tissue volume change and soft tissue linear changes than using a synthetic bioabsorbable membrane alone (negative control group), although these changes were not statistically significant.

Acknowledgements We wish to acknowledge the support from Prof. Fernando Muñoz at the University of Santiago – Lugo Veterinary Hospital where the prophylometric measurements were carried out under his close supervision. We also acknowledge the support of the Biomaterials Group from Universitat Politècnica de Catalunya where the specimens were processed for micro-CT analysis.

Funding information This study was partially funded through a research contract between the Sunstar Group (Switzerland) and the University Complutense of Madrid.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The protocol of the study had been previously approved by the Regional Ethics Committee for Animal Research (EXP-20170327).

Informed consent For this type of study, formal consent is not required.

References

1. Hammerle CHF, Tarnow D (2018) The etiology of hard and soft tissue deficiencies at dental implants: a narrative review. J Clin Periodontol 45(Suppl 20):S267–S277
2. Araujo MG, Lindhe J (2005) Dimensional ridge alterations following tooth extraction. An experimental study in the dog. J Clin Periodontol 32:212–218
3. Tan WL, Wong TLT, Wong MCM, Lang NP (2012) A systematic review of post-extractional alveolar hard and soft tissue dimensional changes in humans. Clin Oral Implants Res 23(Suppl. 5):1–21
4. Moraschini V, Poubel LC, Ferreira V, dos Sp Barboza E (2015) Evaluation of survival and success rates of dental implants reported in longitudinal studies with a follow-up period of at least 10 years: a systematic review. Int J Oral Maxillofac Surg 44:377–388
5. Caneva M, Salata LA, de Souza SS, Baffone G, Lang NP, Botticelli D (2010) Influence of implant positioning in extraction sockets on osseointegration: histomorphometric analyses in dogs. Clin Oral Implants Res 21:43–49
6. Grunder U, Gracis S, Capelli M (2005) Influence of 3-D bone-to-implant relationship on esthetics. Int J Periodontics Restorative Dent 25:113–119
7. Benic GI, Hämmerle CHF (2014) Horizontal bone augmentation by means of guided bone regeneration. Periodontol 66:13–40
8. Sanz-Sánchez I, Carrillo de Albornoz A, Figuero E, Schwarz F, Jung R, Sanz M, Thoma D (2018) Effects of lateral bone augmentation procedures on peri-implant health or disease: a systematic review and meta-analysis. Clin Oral Implants Res 29(Suppl. 15): 18–31
9. Sanz M, Dahlin C, Apatzidou D et al (2019) Biomaterials and regenerative technologies used in bone regeneration in the craniomaxillofacial region: consensus report of group 2 of the 15th European Workshop on Periodontology on Bone Regeneration. J Clin Periodontol 46(Suppl. 21):82–91
10. Haugen HJ, Lyngstadaas SP, Rossi F, Perale G (2019) Bone grafts: which is the ideal biomaterial? J Clin Periodontol 46(Suppl. 21): 92–102
11. Sanz M, Vignoletti F (2015) Key aspects on the use of bone substitutes for bone regeneration of edentulous ridges. Dent Mater 31: 640–647
12. Thoma DS, Bienz SP, Figuero E, Jung RE, Sanz-Martín I (2019) Efficacy of lateral bone augmentation performed simultaneously with dental implant placement: a systematic review and meta-analysis. J Clin Periodontol 46(Suppl. 21):257–276
13. Dahlin C, Linde A, Gottlow J, Nyman S (1988) Healing of bone defects by guided tissue regeneration. Plast Reconstr Surg 81:672–676
14. Omar O, Elgali I, Dahlin C, Thomsen P (2019) Barrier membranes: more than the barrier effect? J Clin Periodontol 46(Suppl. 21):103–123
15. Zitzmann NU, Naef R, Scharer P (1997) Resorbable versus nonresorbable membranes in combination with Bio-Oss for guided bone regeneration. Int J Oral Maxillofac Implants 12(6):844–852
16. Simion M, Baldoni M, Rossi P, Zaffe D (1994) A comparative study of the effectiveness of e-PTFE membranes with and without early exposure during the healing period. Int J Periodontics Restorative Dent 14:167–180
17. Schwarz F, Rothamel D, Herten M, Sager M, Becker J (2006) Angiogenesis pattern of native and cross-linked collagen membranes: an immunohistochemical study in the rat. Clin Oral Implants Res 17:403–409
18. Schwarz F, Rothamel D, Herten M, Sager M, Ferrari D, Becker J (2008) Immunohistochemical characterization of guided bone regeneration at dehiscence-type defect using different barrier membranes: an experimental study in dogs. Clin Oral Implants Res 19: 402–415
19. Rothamel D, Schwarz F, Sager M, Herten M, Becker J (2005) Biodegradation of differently cross-linked collagen membranes: an experimental study in the rat. Clin Oral Implants Res 16:369–378
20. Gentile P, Chiono V, Tonda-Turo C, Ferreira AM, Ciardelli G (2011) Polymeric membranes for guided bone regeneration. Biotechnol J 6(10):1187–1197
21. Gottlow J, Laurell L, Lundgren D, Mathisen T, Nyman S, Rylander H, Bogentoff C (1994) Periodontal tissue response to a new bioresorbable guided tissue regeneration device: a longitudinal study in monkeys. Int J Periodontics Restorative Dent 14:437–449
22. Lundgren D, Laurell L, Gottlow J, Rylander H, Torbjörn M, Nyman S, Rask M (1995) The influence of the design of two different bioresorbable barriers on the results of guided tissue regeneration therapy. An intra-individual comparative study in the monkey. J Periodontol 66:605–612
23. Matsumoto G, Hoshino J, Kinoshita Y, Sugita Y, Kubo K, Maeda H, Ikada Y, Kinoshita Y (2012) Alveolar bone regeneration using poly-(lactic acid-co-glycolic acid-co-e-caprolactone) porous membrane with collagen sponge containing basic fibroblast growth factor: an experimental study in the dog. J Biomater Appl 27(4):485–493
24. Won JY, Park CY, Bae JH, Ahn G, Kim C, Lim DH, Cho DW, Yun WS, Shim JH (2016) Evaluation of 3D printed PCL/PLGA/-TCP versus collagen membranes for guided bone regeneration in a beagle implant model. Biomed Mater 11(5):055013
25. Sanz-Martın I, Sailer I, Hammerle CHF, Thoma DS (2016) Soft tissue stability and volumetric changes after 5 years in pontic sites with or without soft tissue grafting: a retrospective cohort study. Clin Oral Implants Res 27:969–974
26. Sanz-Martin I, Ferrantino L, Vignoletti F, Nunez J, Baldini N, Duvina M, Alcaraz J, Sanz M (2017) Contour changes after guided

bone regeneration of large non-contained mandibular buccal bone defects using deproteinized bovine bone mineral and a porcine-derived collagen membrane: an experimental in vivo investigation. Clin Oral Investig 22(3):1273–1283
27. Sanz Martin I, Vignoletti F, Nuñez J, Permuy M, Muñoz F, Sanz-Esporrin J, Fierravanti L, Shapira L, Sanz M (2017) Hard and soft tissue integration of immediate and delayed implants with a modified coronal macro design: histological, micro CT and volumetric soft tissue changes from a pre-clinical in vivo study. J Clin Periodontol 44(8):842–853
28. Basler T, Naenni N, Schneider D, Hammerle CHF, Jung R, Thoma DS (2018) Randomized controlled clinical study assessing two membranes for guided bone regeneration of peri-implant bone defects: 3-year results. Clin Oral Implants Res 29(5):499–507
29. Di Raimondo R, Sanz-Esporrin J, Pla R, Sanz-Martin I, Luengo F, Vignoletti F, Nuñez J, Sanz M (2019) Alveolar crests contour changes after guided bone regeneration using different biomaterials: an experimental in vivo investigation. Clin Oral Investig. https://doi.org/10.1007/s00784-019-03092-8
30. Khobragade P, Jain A, Setlur Nagesh SV, Andreana S, Dziak R, Sunkara SK, Ionita CN (2015) Micro-computed tomography (CT) based assessment of dental regenerative therapy in the canine mandible model. Proc SPIE Int Soc Opt Eng 17:9417
31. Baek YJ, Kim JH, Song JM, Zoon SY, Kim HS, Shin SH (2016) Chitin-fibroin-hydroxyapatite membrane for guided bone regeneration: micro-computed tomography evaluation in a rat model. Maxillofac Plast Reconstr Surg 38(1):14
32. Vignoletti F, Abrahamsson I (2012) Quality of reporting of experimental research in implant dentistry. Critical aspects in design, outcome assessment and model validation. J Clin Periodontol 39(Suppl. 12):6–27
33. Song JM, Shin SH, Kim YD, Lee JY, Baek YJ, Yoon SY, Kim HS (2014) Comparative study of chitosan/fibroin–hydroxyapatite and collagen membranes for guided bone regeneration in rat calvarial defects: micro-computed tomography analysis. Int J Oral Sci 6(2):87–93
34. Sanz M, Ferrantino L, Vignoletti F, de Sanctis M, Berglundh T (2017) Guided bone regeneration of non-contained mandibular buccal bone defects using deproteinized bovine bone mineral and a collagen membrane: an experimental in vivo investigation. Clin Oral Implants Res 28:1466–1476
35. Hoornaert A, d'Arros C, Heymann MF, Layrolle P (2016) Biocompatibility, resorption and biofunctionality of a new synthetic biodegradable membrane for guided bone regeneration. Biomed Mater 11(4):045012
36. Lundgren D, Sennerby L, Falk H, Friberg B, Nyman S (1994) The use of a new bioresorbable barrier for guided bone regeneration in connection with implant installation. Case reports. Clin Oral Implants Res 5:177–184
37. Thoma DS, Jung U-W, Park J-Y, Bienz SP, Husler J, Jung RE (2017) Bone augmentation at peri-implant dehiscence defects comparing a synthetic polyethylene glycol hydrogel matrix versus standard guided bone regeneration techniques. Clin Oral Implants Res 28:e76–e83
38. Jung UW, Cha JK, Vignoletti F, Nunez J, Sanz J, Sanz M (2017) Simultaneous lateral bone augmentation and implant placement using a particulated synthetic bone substitute around chronic peri-implant dehiscence defects in dogs. J Clin Periodontol 44:1172–1180
39. Bienz SP, Jung RE, Sapata VM, Hammerle CHF, Husler J, Thoma DS (2017) Volumetric changes and peri-implant health at implant sites with or without soft tissue grafting in the esthetic zone, a retrospective case-control study with a 5-year follow-up. Clin Oral Implants Res 28:1459–1465
40. Antunes AA, Grossi-Oliveira GA, Martins-Neto EC, Almeida AL, Salata LA (2015) Treatment of circumferential defects with osseoconductive xenografts of different porosities: a histological, histometric, resonance frequency analysis, and micro-CT study in dogs. Clin Implant Dent Relat Res 17(Suppl 1):e202–e220
41. Sanz-Martin I, Permuy M, Vignoletti F, Nuñez J, Muñoz F, Sanz M (2018) A novel methodological approach using superimposed micro-CT and STL images to analyze hard and soft tissue volume in immediate and delayed implants with different cervical designs. Clin Oral Implants Res 29:986–995

***Artículo 3**: Hard tissue volumetric and soft tissue contour linear changes at implants with different surface characteristics after experimentally induced peri-implantitis. An experimental in vivo investigation. Di Raimondo R, Sanz-Esporrin J, Sanz-Martin I, Vignoletti F, Nuñez J, Muñoz F, Haugen HJ, Sanz M. (Accepted with minor changes)*

Objetivo. Comparar en un modelo de peri-implantitis experimental, los cambios lineales del contorno vestibular y lingual de los tejidos blandos y los cambios volumétricos de los tejidos duros, de implantes con el mismo diseño y macroestructura, pero con dos superficies diferentes.

Material y métodos. El estudio se puede dividir en tres períodos: fase preparatoria, inducción activa de la peri-implantitis y fase de progresión espontánea. En la fase preparatoria se realizaron bilateralmente la hemisección y extracciones de los dientes (Pm2, Pm3, Pm4 y M1) *(Imagen Suplementaria 4)*, y después de 3 meses se colocaron en las crestas cicatrizadas cinco implantes por cada hemi-mandíbula. i) Los implantes del grupo test presentaban en su exterior una capa de multi-fosfonatos; ii) los implantes del grupo control no presentaban ningún tratamiento de superficie. Después de 3 meses en los cuales los implantes se osteointegraron, se interrumpió cualquier medida de higiene y se procedió con la fase de inducción activa de la peri-implantitis, en la cual se colocaron ligaduras de algodón en una posición subgingival alrededor de todos los implantes, además de sustituirlas cada 4 semanas por un total de 4 meses, momento en el cual se retiraron dichas ligaduras y se dejó progresar la peri-implantitis de manera espontánea durante otros 3 meses.

Resultados. El análisis de los tejidos duros mostró una marcada pérdida de hueso alrededor de ambas superficies de implantes (test y control), sin diferencias significativas entre los grupos. En el análisis de los tejidos blandos se observó un aumento del contorno horizontal en la fase de inducción activa y una reducción del mismo durante la fase de progresión espontánea, y sin diferencias significativas entre los grupos. Además, a nivel vertical se observaron dehiscencias

de los tejidos blandos que fueron mayores en el aspecto vestibular con respecto a la parte lingual, aunque las diferencias entre los dos grupos no fueron tampoco significativas.

Conclusiones: La superficie de los implantes con los fosfonatos (grupo test) no proporciona ventajas evidentes frente a la progresión de la peri-implantitis experimental, en términos de cambios del contorno de los tejidos blandos y pérdida de hueso tridimensional.

Clinical Oral Investigations

HARD TISSUE VOLUMETRIC AND SOFT TISSUE CONTOUR LINEAR CHANGES AT IMPLANTS WITH DIFFERENT SURFACE CHARACTERISTICS AFTER EXPERIMENTALLY INDUCED PERI-IMPLANTITIS. AN EXPERIMENTAL IN VIVO INVESTIGATION

--Manuscript Draft--

Manuscript Number:	CLOI-D-20-01998R1
Full Title:	HARD TISSUE VOLUMETRIC AND SOFT TISSUE CONTOUR LINEAR CHANGES AT IMPLANTS WITH DIFFERENT SURFACE CHARACTERISTICS AFTER EXPERIMENTALLY INDUCED PERI-IMPLANTITIS. AN EXPERIMENTAL IN VIVO INVESTIGATION
Article Type:	Original Article
Corresponding Author:	Javier Sanz-Esporrin Universidad Complutense de Madrid Facultad de Odontologia SPAIN
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Universidad Complutense de Madrid Facultad de Odontologia
Corresponding Author's Secondary Institution:	
First Author:	Riccardo Di Raimondo
First Author Secondary Information:	
Order of Authors:	Riccardo Di Raimondo
	Javier Sanz-Esporrin
	Ignacio Sanz-Martin
	Fabio Vignoletti
	Javier Nuñez
	Fernando Muñoz
	Håvard Jostein Haugen
	Mariano Sanz
Order of Authors Secondary Information:	
Funding Information:	
Abstract:	Objective To evaluate the hard tissue volumetric and soft tissue contour linear changes in implants with two different implant surface characteristics after a ligature induced peri-implantitis Material and Methods In eight beagle dogs, implants with the same size and diameter but distinct surfaces characteristics were placed in the healed mandibular sites. Test implants had an external monolayer of multi-phosphonate molecules (B+), while control implants were identical but without the phosphonate-rich surface. Once the implants were osseointegrated, oral hygiene was interrupted and peri-implantitis was induced by placing subgingival ligatures. After 16 weeks, the ligatures were removed and peri-implantitis progressed spontaneously. Bone to implant contact (BIC) and bone loss (BL) were assessed three-dimensionally with Micro-Ct (µCT). Dental casts were optically scanned and the obtained digitalized standard tessellation language (STL)

	images were used to assess the soft tissue vertical and horizontal contour linear changes. Results Reduction of the three-dimensional BIC percentage during the induction and progression phases of the experimental peri-implantitis was similar for both the experimental and control implants, without statistically significant differences between them. Soft tissue analysis revealed for both implant groups an increase in horizontal dimension after the induction of peri-implantitis, followed by a decrease after the spontaneous progression period. In the vertical dimension a soft tissue dehiscence was observed in both groups, being more pronounced at the buccal aspect. Conclusions The added phosphonate-rich surface did not provide a more resistant environment against experimental peri-implantitis, when assessed by the changes in bone volume and soft tissue contours. Clinical relevance Ligature induced peri-implantitis is a validated model to study the tissue changes occurring during peri-implantitis. It was hypothesized that a stronger osseointegration mediated by the chemical bond of a phosphonate rich implant surface would develop an environment more resistant to the inflammatory changes occurring after experimental peri-implantitis. These results, however, indicate that the hard and soft tissue destructive changes occurring at both the induction and progression phases of experimental peri-implantitis were not influenced by the quality of osseointegration.
Response to Reviewers:	CLOI-D-20-01998 Comments of the author This revised manuscript has been corrected following the recommendations and helpful comments from the reviewers. In this revised version the changes are marked in red and bold characters. Referee’s Answer Reviewers' comments: •Reviewer # 1 1.Abstarct. Clinical Relevance: # Page 2 line 49: In the sentence "These results, however, indicate that the hard and tissue destructive change occurring at both the induction and progression phases…". Did the authors mean hard and soft tissue destructive changes? If yes, please correct this sentence. ANSWER: We have corrected the sentence following to reviewer’s suggestion, since we referred to both soft and hard tissues. 2.Introduction: # Page 3 line 22: In the sentence: "In its incidence and progression, there are well established risk, such as the patient's history of periodontitis…". Did the authors mean there are well established risk indicators? If yes, please complete this sentence. ANSWER: We have modified the sentence. In this context, while history of periodontitis, oral hygiene practices and lack of compliance with maintenance therapy are considered risk factors, those associated with the implant are considered risk indicators.

Page 4 line 1: In the sentence: "…after experimental peri-implantitis using Micro-Ct volumetric analysis to assess the bone changes and digitized standard tessellation language (STL) images to assess the soft tissue contour lineal changes."

* Please replace the word "digitized" by the word "digitalized".
* Please be consistent with the abbreviation of STL, which differs from other sections (abstract and body of the paper).

ANSWER: We have corrected the word “digitalized”, as well as the abbreviation of “STL” in all the sections of the manuscript (abstract, M&M), according to reviewer’s suggestion.

3.Material and Methods

* Volumetric hard tissue analysis: Micro-Ct image acquisition and data analysis
Please describe who performed the volumetric hard tissue analysis, and how was the investigator(s) calibration performed before the data analysis.

ANSWER: We have added the name of the investigator who performed the volumetric hard tissue analysis (JSE) and we have clarified in the manuscript the calibration process.

* Soft tissue contour analysis: Stereolithography (STL) image and data analysis
# Page 6 line 60: The authors refer that "further points of reference were selected manually, until achieving a "fine fit superimposition".

ANSWER: We appreciate the reviewer’s consideration and we have tried to clarify this issue as follows: to attain a “fine fit superimposition” we selected a minimum of 10 points, following the protocol described in the publication by Becker et al. 2018 (Becker K, Wilmes B, Grandjean C, Drescher D (2018) Impact of manual control point selection accuracy on automated surface matching of digital dental models. Clin Oral Investig.; 22(2):801-810). We have modified the sentence in the body of the manuscript (M & M) and we have added that these further reference points were at least 10, as well as we have added this reference (Becker et al. 2018).

* Please could the authors describe how did they establish the superimposition was fine?

ANSWER: We have tried to clarify this issue as follows: since the software used to superimpose the models works by mathematical algorithms superimposing the maximum number of points, this software establishes when the appropriate superimposition has been achieved.

* Did the software provide any measurement or percentage (%) of error between the superimpositions and between the different number of reference points that were selected?

ANSWER: to clarify this issue we have added a sentence at the end of the “limitation section”, in which we specify the percentage (%) of error of this method of analysis, as well as the coefficient of variation between measurements, according to the article of Mehl et al. 1997 (Mehl A, Gloger W, Kunzelmann KH, Hickel R (1997) A new optical 3-D device for the detection of wear. J Dent Res.;76 (11): 1799-807) and the article of Windisch et al. 2007 (Windisch SI, Jung, RE, Sailer I, Studer SP, Ender A, Hämmerle CHF (2007) A new optical method to evaluate three-dimensional volume changes of alveolar contours: a methodological in vitro study. Clin Oral Implants Res.; 18: 545-551).
Moreover, we have also added these two references in the manuscript.

* Please describe why did you select 5 reference points and then further points?

ANSWER: We have tried to clarify this issue as follows: In order to attain an

	appropriate matching, we first selected three to five fixed reference points to provide the software a "rough fit" superimposition. Then, as mentioned previously, a minimum of 10 more reference points was added to achieve a “fine fit superimposition” (Becker et al. 2018) * Obs: The number of reference points at each implant site should be standard throughout the analysis for consistency in the data acquisition. ANSWER: Indeed this “standard” was a minimum of 10 number of points, as mentioned previously. # Page 7 line 1: The authors describe that a calibrated investigator performed the soft tissue contour linear analysis. *How was the calibration performed? *Was an intra-examiner calibration done? *Please provide the intraclass correlation coefficient values. ANSWER: We have tried to clarify these questions as follows. Through several years of training performing soft tissue contour lineal analysis, the primary author of this manuscript (RDR) has developed expertise and precision, always under the supervision of a senior investigator (ISM), also expert in soft tissue analysis. However, an actual calibration exercise was not performed in this study, and as consequence we have changed in the manuscript the word “calibrated” with the word “trained”. 4.Results: * Regarding the vertical linear changes of soft tissue at both buccal and lingual aspects, table 4 shows that at control and test groups the major changes occurred at buccal aspects mainly. Did the authors perform any statistical analysis to compare the linear changes between buccal and lingual sites? Please provide this data in the results section. ANSWER: Following the advice from the reviewer, we have modified table 4 including the differences between the buccal and lingual measurements in each group and the corresponding p-values. * Please provide some graphs (i.e. bar, linear) for a better and clearer understanding of the results presented in the tables. There are too many values which are hard to understand at a first glance. ANSWER: Following the advice from the reviewer, we have added 4 graphs to better describe the results for both the soft and hard tissues. These graphs are referenced in the manuscript as “Supplementary graphs 1, and graphs 1, 2, 3” 5. Discussion: * Please include the methodological limitations of the study. Volumetric analysis and scan evaluations do have certain methodological confines. Please describe them. ANSWER: Following the advice from the reviewer, we have added a new paragraph at the end of the manuscript with a section related to the methodological limitations of the study. * Please describe the limitations of a ligature induced model. ANSWER: Following the advice from the reviewer, we have added a new paragraph at the end of the manuscript with a section related to the ligature induced model limitations of the study, * Please further discuss the possible reasons (i.e. inflammation) of why the soft tissue

	contour changes could be increased and decreased over the different phases of the study. ANSWER: Following the advice from the reviewer, we have added a new paragraph at the beginning of the discussion section discussing the reasons of the increase and decrease of contour on the respective induction and progression phases. •Reviewer # 2: 1.Authors assume or is there any evidence that a Phosphonate rich implant surface achieves a higher degree of osseointegration (BIC, torque removal)? ANSWER: There is evidence from preclinical investigations that this surface coating provides improved osteointegration. These investigations are properly referenced in the manuscript (Von Salis-Soglio et al. 2014) 2.Please modify this sentence: `indicate that the hard and tissue destructive changes´ ANSWER: We have corrected the sentence, according to reviewer's suggestion. 3.Was the spontaneous progression phase of enough time according to the existing scientific evidence? ANSWER: We have carried out a timing for this phase, which proved to show spontaneous progression in previous preclinical investigations within our research group (Sanz-Esporrin et al. 20199. This timing, between 2 and 6 months of progression phase has also been utlisied by other research groups using similar experimental designs (Berglundh et al. 2007; Albouy et al. 2008). 4.16 implants were lost intentionally or as a failure in the methodology? ANSWER: As stated in the first part of the "Results section", the most mesial implant of each hemi-mandible (16 implants in total) was excluded from the analysis, since a dehiscence defect was created intentionally during the implant placement surgery, and therefore the analysis of these implants was not included in the present report. 5.Then, 17 implants were excluded for soft tissue measurements. Could you have done this investigation with less dogs? ANSWER: The analysis of the soft tissues was performed in all dogs (8 dogs), but due to imperfections in the obtained cast models in 17 implants) (8 test and 9 control) we discarded them and hence we have analyzed 47 implants (24 test and 23 control), in which no imperfections were observed. To help the readers in better understanding who the soft tissue analysis was carried out, we have modified some sentences in the first part of the "Results section" 6.Please correct: the lack of histological results in this report may limit a a true understanding of the tissue behaviour. ANSWER: We have corrected the sentence, according to reviewer's suggestion. 7.Peri-implantitis is measured by PPD and radiographic bone loss measured with PA Rx. Is this investigation assessing peri-implantitis? ANSWER: We appreciate the reviewer's consideration, and we clarify this point as follows. In this publication we present a novel method to evaluate the soft and hard tissue changes occurring during peri-implantitis. However, an additional objective was to study the influence of this bioactive surface (Phosphonate rich implant surface) in

	preventing the bone changes occurring during experimental peri-implantitis. These results will be published in an independent report.
Manuscript Classifications:	4.1: CBCT imaging; 8.6: Implant dentistry; 8.7: Peri-Implantitis; 8.9: Preclinical studies in periodontology and implant dentistry

"**Letter of response**"

TITLE: "HARD TISSUE VOLUMETRIC AND SOFT TISSUE CONTOUR LINEAR CHANGES AT IMPLANTS WITH DIFFERENT SURFACE CHARACTERISTICS AFTER EXPERIMENTALLY INDUCED PERI-IMPLANTITIS. AN EXPERIMENTAL IN VIVO INVESTIGATION" (Riccardo Di Raimondo et. al)

CLOI-D-20-01998

Comments of the author
This revised manuscript has been corrected following the recommendations and helpful comments from the reviewers.

In this revised version the changes are marked in red and bold characters.

Referee's Answer

Reviewers' comments:
- Reviewer # 1

1. Abstarct. Clinical Relevance:

Page 2 line 49: In the sentence "These results, however, indicate that the hard and tissue destructive change occurring at both the induction and progression phases…". Did the authors mean hard and soft tissue destructive changes? If yes, please correct this sentence.

ANSWER: We have corrected the sentence following to reviewer's suggestion, since we referred to both soft and hard tissues.

2. Introduction:

Page 3 line 22: In the sentence: "In its incidence and progression, there are well established risk, such as the patient's history of periodontitis…". Did the authors mean there are well established risk indicators? If yes, please complete this sentence.

ANSWER: We have modified the sentence. In this context, while history of periodontitis, oral hygiene practices and lack of compliance with maintenance therapy are considered risk factors, those associated with the implant are considered risk indicators.

Page 4 line 1: In the sentence: "…after experimental peri-implantitis using Micro-Ct volumetric analysis to assess the bone changes and digitized standard tessellation language (STL) images to assess the soft tissue contour lineal changes."

* Please replace the word "digitized" by the word "digitalized".
* Please be consistent with the abbreviation of STL, which differs from other sections (abstract and body of the paper).

ANSWER: We have corrected the word "digitalized", as well as the abbreviation of "STL" in all the sections of the manuscript (abstract, M&M), according to reviewer's suggestion.

3. Material and Methods

* Volumetric hard tissue analysis: Micro-Ct image acquisition and data analysis
Please describe who performed the volumetric hard tissue analysis, and how was the investigator(s) calibration performed before the data analysis.

ANSWER: We have added the name of the investigator who performed the volumetric hard tissue analysis (JSE) and we have clarified in the manuscript the calibration process.

* Soft tissue contour analysis: Stereolithography (STL) image and data analysis
# Page 6 line 60: The authors refer that "further points of reference were selected manually, until achieving a "fine fit superimposition".

ANSWER: We appreciate the reviewer's consideration and we have tried to clarify this issue as follows: to attain a "fine fit superimposition" we selected a minimum of 10 points, following the protocol described in the publication by Becker et al. 2018 (Becker K, Wilmes B, Grandjean C, Drescher D (2018) Impact of manual control point selection accuracy on automated surface matching of digital dental models. Clin Oral Investig.; 22(2):801-810). We have modified the sentence in the body of the manuscript (M & M) and we have added that these further reference points were at least 10, as well as we have added this reference (Becker et al. 2018).

* Please could the authors describe how did they establish the superimposition was fine?

ANSWER: We have tried to clarify this issue as follows: since the software used to superimpose the models works by mathematical algorithms superimposing the maximum number of points, this software establishes when the appropriate superimposition has been achieved.

* Did the software provide any measurement or percentage (%) of error between the superimpositions and between the different number of reference points that were selected?

ANSWER: to clarify this issue we have added a sentence at the end of the "limitation section", in which we specify the percentage (%) of error of this method of analysis, as well

as the coefficient of variation between measurements, according to the article of Mehl et al. 1997 (Mehl A, Gloger W, Kunzelmann KH, Hickel R (1997) A new optical 3-D device for the detection of wear. J Dent Res.;76 (11): 1799-807) and the article of Windisch et al. 2007 (Windisch SI, Jung, RE, Sailer I, Studer SP, Ender A, Hämmerle CHF (2007) A new optical method to evaluate three-dimensional volume changes of alveolar contours: a methodological in vitro study. Clin Oral Implants Res.; 18: 545-551).
Moreover, we have also added these two references in the manuscript.

* Please describe why did you select 5 reference points and then further points?

ANSWER: We have tried to clarify this issue as follows: In order to attain an appropriate matching, we first selected three to five fixed reference points to provide the software a "rough fit" superimposition. Then, as mentioned previously, a minimum of 10 more reference points was added to achieve a "fine fit superimposition" (Becker et al. 2018)

* Obs: The number of reference points at each implant site should be standard throughout the analysis for consistency in the data acquisition.

ANSWER: Indeed this "standard" was a minimum of 10 number of points, as mentioned previously.

Page 7 line 1: The authors describe that a calibrated investigator performed the soft tissue contour linear analysis.
*How was the calibration performed?
*Was an intra-examiner calibration done?
*Please provide the intraclass correlation coefficient values.

ANSWER: We have tried to clarify these questions as follows.
Through several years of training performing soft tissue contour lineal analysis, the primary author of this manuscript (RDR) has developed expertise and precision, always under the supervision of a senior investigator (ISM), also expert in soft tissue analysis. However, an actual calibration exercise was not performed in this study, and as consequence we have changed in the manuscript the word "calibrated" with the word "trained".

4. Results:

* Regarding the vertical linear changes of soft tissue at both buccal and lingual aspects, table 4 shows that at control and test groups the major changes occurred at buccal aspects mainly. Did the authors perform any statistical analysis to compare the linear changes between buccal and lingual sites? Please provide this data in the results section.

ANSWER: Following the advice from the reviewer, we have modified table 4 including the differences between the buccal and lingual measurements in each group and the corresponding p-values.

* Please provide some graphs (i.e. bar, linear) for a better and clearer understanding of the results presented in the tables. There are too many values which are hard to understand at a first glance.

ANSWER: Following the advice from the reviewer, we have added 4 graphs to better describe the results for both the soft and hard tissues. These graphs are referenced in the manuscript as "Supplementary graphs 1, and graphs 1, 2, 3"

5. Discussion:

* Please include the methodological limitations of the study. Volumetric analysis and scan evaluations do have certain methodological confines. Please describe them.

ANSWER: Following the advice from the reviewer, we have added a new paragraph at the end of the manuscript with a section related to the methodological limitations of the study.

* Please describe the limitations of a ligature induced model.

ANSWER: Following the advice from the reviewer, we have added a new paragraph at the end of the manuscript with a section related to the ligature induced model limitations of the study,

* Please further discuss the possible reasons (i.e. inflammation) of why the soft tissue contour changes could be increased and decreased over the different phases of the study.

ANSWER: Following the advice from the reviewer, we have added a new paragraph at the beginning of the discussion section discussing the reasons of the increase and decrease of contour on the respective induction and progression phases.

- Reviewer # 2:

1. Authors assume or is there any evidence that a Phosphonate rich implant surface achieves a higher degree of osseointegration (BIC, torque removal)?

ANSWER: There is evidence from preclinical investigations that this surface coating provides improved osteointegration. These investigations are properly referenced in the manuscript (Von Salis-Soglio et al. 2014)

2. Please modify this sentence: `indicate that the hard and tissue destructive changes´

ANSWER: We have corrected the sentence, according to reviewer's suggestion.

3. Was the spontaneous progression phase of enough time according to the existing scientific evidence?

ANSWER: We have carried out a timing for this phase, which proved to show spontaneous progression in previous preclinical investigations within our research group (Sanz-Esporrin et al. 20199. This timing, between 2 and 6 months of progression phase has also been utlisied by other research groups using similar experimental designs (Berglundh et al. 2007; Albouy et al. 2008).

4. 16 implants were lost intentionally or as a failure in the methodology?

ANSWER: As stated in the first part of the "Results section", the most mesial implant of each hemi-mandible (16 implants in total) was excluded from the analysis, since a dehiscence defect was created intentionally during the implant placement surgery, and therefore the analysis of these implants was not included in the present report.

5. Then, 17 implants were excluded for soft tissue measurements. Could you have done this investigation with less dogs?

ANSWER: The analysis of the soft tissues was performed in all dogs (8 dogs), but due to imperfections in the obtained cast models in 17 implants) (8 test and 9 control) we discarded them and hence we have analyzed 47 implants (24 test and 23 control), in which no imperfections were observed. To help the readers in better understanding who the soft tissue analysis was carried out, we have modified some sentences in the first part of the "Results section"

6. Please correct: the lack of histological results in this report may limit a a true understanding of the tissue behaviour.

ANSWER: We have corrected the sentence, according to reviewer's suggestion.

7. Peri-implantitis is measured by PPD and radiographic bone loss measured with PA Rx. Is this investigation assessing peri-implantitis?

ANSWER: We appreciate the reviewer's consideration, and we clarify this point as follows. In this publication we present a novel method to evaluate the soft and hard tissue changes occurring during peri-implantitis. However, an additional objective was to study the

influence of this bioactive surface (Phosphonate rich implant surface) in preventing the bone changes occurring during experimental peri-implantitis. These results will be published in an independent report.

HARD TISSUE VOLUMETRIC AND SOFT TISSUE CONTOUR LINEAR CHANGES AT IMPLANTS WITH DIFFERENT SURFACE CHARACTERISTICS AFTER EXPERIMENTALLY INDUCED PERI-IMPLANTITIS. AN EXPERIMENTAL IN VIVO INVESTIGATION

Authors:

Di Raimondo R[1], Sanz-Esporrín J[2], Sanz Martin I[1], Vignoletti F[2], NuñezJ[1], Muñoz F[3], Haugen HJ[4], Sanz M[1,2]

Institutions:

1. Graduate Clinic of Periodontology, Faculty of Odontology, University Complutense, Madrid, Spain.
2. ETEP (Aetiology and Therapy of Periodontal and Peri-Implant Diseases) Research Group, University Complutense, Madrid, Spain.
3. Department of Veterinary Clinical Sciences, University of Santiago de Compostela, Lugo, Spain.
4. Department of Biomaterials, Institute for Clinical Dentistry, University of Oslo, Norway.

Running title: Contour tissue changes after experimental peri-implantitis

Corresponding author:

Javier Sanz-Esporrin

Facultad de Odontología – Universidad Complutense de Madrid

Plaza Ramón y Cajal s/n (Ciudad Universitaria)

28040 Madrid

SPAIN

e-mail: javisanzes@gmail.com

telephone number: +34 913942021

Acknowledgements

The authors would like to express their gratitude to Liebert Parreiras Nogueira at the Clinical Oral Research Laboratory, Faculty of Dentistry, University of Oslo for their help preparing and analysing the microCT data.

Abstract

Objective: To evaluate the hard tissue volumetric and soft tissue contour linear changes in implants with two different implant surface characteristics after a ligature induced peri-implantitis

Material and Methods: In eight beagle dogs, implants with the same size and diameter but distinct surfaces characteristics were placed in **the** healed mandibular sites. Test implants had an external monolayer of multi-phosphonate molecules (B+), while control implants were identical but without the phosphonate-rich surface. Once the implants were osseointegrated, oral hygiene was interrupted and peri-implantitis was induced by placing subgingival ligatures. After 16 weeks, **the** ligatures were removed and peri-implantitis progressed spontaneously. Bone to implant contact (BIC) and bone loss (BL) were assessed three-dimensionally with Micro-Ct **(μCT)**. Dental casts were optically scanned and the obtained **digitalized standard tessellation language (STL) images** were used to assess the soft tissue vertical and horizontal contour linear changes.

Results: **Reduction of the** three-dimensional BIC **percentage during the induction and progression phases of the experimental peri-implantitis was similar for both the** experimental and control implants, without statistically significant differences between them. **Soft** tissue analysis **revealed for** both implant groups an increase in horizontal dimension after the induction of peri-implantitis, followed by a decrease after the spontaneous progression period. In the vertical dimension a soft tissue dehiscence was observed in both groups, being more pronounced at the buccal aspect.

Conclusions: The added phosphonate-rich surface did not provide a more resistant environment against experimental peri-implantitis, when assessed by the changes in bone volume and soft tissue contours.

Clinical relevance: Ligature induced peri-implantitis is a validated model to study the tissue changes occurring **during** peri-implantitis. It was hypothesized that a stronger osseointegration mediated by the chemical bond of a phosphonate rich implant surface would develop an environment more resistant to the inflammatory changes occurring after experimental peri-implantitis. These results, however, indicate that the hard and **soft** tissue destructive changes occurring at both the induction and progression phases of experimental peri-implantitis were not influenced by the quality of osseointegration.

Keywords: experimental in vivo investigation, implant surface microtopography, experimental peri-implantitis, Micro-CT analysis, volumetric analysis.

Introduction

Oral rehabilitation with dental implants after tooth extraction is the current standard of care, with demonstrated high long-term survival rates (94.6% after 10 years) [1]. However, in spite of these high survival rates, a high incidence of both technical and biological complications has been reported [2]. At the World Workshop of Periodontology, a new classification of peri-implant diseases was introduced [3, 4] defining peri-implantitis as a pathological condition caused by biofilm accumulation on the implant supported restoration and characterized by inflammation of the peri-implant soft tissues and progressive bone loss [5]. Prevalence of peri-implantitis has been estimated between 15 and 25% at patient level, although these figures vary depending on the different populations assayed, the long-term evaluation and the threshold of bone level changes used to define the cases [6, 7]. Although this disease shares a similar etiology and pathogenesis with periodontitis, it has shown a more rapid and advanced progression [5, 8, 9]. In its incidence and progression, there are well established risk factors, such as the patient's history of periodontitis and its oral hygiene practices and lack of compliance with maintenance therapy [5, 10, 11], however, there are other risk indicators associated with the implant itself and implant site that have not yet been validated in prospective cohort studies [4]. Among them, the possible influence of the implant design, mainly the implant surface topographic characteristics have been evaluated in recent systematic reviews. Although rougher compared to smoother surfaces seem to favor plaque accumulation and hence peri-implantitis, there is a high discrepancy among the results from different investigations [12, 13].

Different investigators have hypothesized that by changing the chemical composition of the implant surface design, the quality of osseointegration might increase and lead to a stronger higher bone to implant contact (BIC), what might decrease the incidence of peri-implantitis [14]. This was not the case of calcium phosphate coatings that failed to demonstrate a higher degree of osseointegration when compared to standard implants surfaces of pure titanium [15, 16]. The addition of an external layer of multi-phosphonate molecules to the traditional implant surface was designed for increasing the implant wettability and thus, favoring a faster osteoblastic activity and a stronger bond between the bone and the implant surface [17, 18]. Histological preclinical in vivo investigations showed that this surface modification could accelerated the bone healing process by promoting faster bone formation and osseointegration [19]. Clinically, this multiphosphonate coated surface (SurfLink®, Nano Bridging Molecules, Gland, Switzerland) has been evaluated against a standard roughened implant surface, but results did not provide a significant benefit compared with standard implants and the authors suggested that this surface should be tested under more critical conditions [20]. One of these critical conditions may be to evaluate the resistance of this bioactive surface when exposed to the etiological conditions leading to peri-implantitis. It was therefore, the aim of this preclinical in vivo investigation to evaluate the behavior of this novel implant surface by assessing the peri-implant hard and soft tissue changes after experimental peri-implantitis using μCT volumetric analysis to assess the bone changes and digitalized standard tessellation language (STL) images to assess the soft tissue contour lineal changes.

Material and Methods

Study design

This preclinical in vivo investigation was designed according to the modified ARRIVE guidelines [21]. The protocol of this investigation was approved by the Regional Ethics Committee for Animal Research of the University of Santiago de Compostela (Ref. AE-LU-001/04/16) assuring the compliance with the Spanish regulation RD53/2013 for preclinical investigations, including the handling, physiological conditions, health care, nutrition and housing of the experimental animals used in the investigation.

All surgical procedures and soft tissue contour linear changes analysis were performed at the Department of Veterinary, in the University of Lugo, Spain. The **μCT** data acquisition and 3D hard tissue volumetric analysis were performed in the Department of Biomaterials, University of Oslo.

The study population consisted on eight female adult Beagle dogs acquired from the Service of Animal Experimentation of the University of Cordoba, Spain, with a mean age of 72 months, weighting between 12 and 15 kg. Each experimental animal was maintained in individual kennels in a 12:12 light/dark cycle and 22-21°C and monitored daily by an experienced veterinarian, being each identified with a subcutaneous chip code that remained during entire follow-up of the study. Food was based on soft pallet diet and the animals had free access to water. Before final inclusion for the investigation, all animals were observed 2 weeks prior to the surgical procedures to assure their general health status.

Surgical interventions

All surgical procedures were carried out between June 2016 and July 2017. The study could be divided in three different time periods: a) the preparatory phase (from June and November 2016); b) the induction period of peri-implantitis (from November 2016 and February 2017) and c) the spontaneous progression of peri-implantitis until euthanasia (from February and July 2017).

Immediately before each surgery, the animals were sedated with propofol (2mg/kg/i.v., Propovet, Abbott Laboratories, Kent, UK) and placed under general anesthesia with 2.5-4% of isoflurane (Isoba-vet, Schering-Plough, Madrid Spain) for the entire period of the surgery. Lidocaine 2% with epinephrine 1:100.000 (2% Xylocaine Dental, Dentsply, York, PA, USA) was also infiltrated locally to reduce intra-operatory bleeding. The preparatory phase included two different periods of three months each. In the first surgery, the second, third and fourth lower premolars (P2, P3, P4) and the first molar (M1) were extracted once hemisected (Fig. 1a) (Fig. 1b). The extraction sockets were left to heal spontaneously during three months, during which time the oral hygiene **of the dogs** was maintained professionally, using soft toothbrushes and toothpaste, and by applying a gauze impregnated with a Chlorhexidine solution (0.12%), three times per week.

After this period, the second surgery consisted in the elevation of full thickness flaps to expose the healed edentulous ridges (Fig. 1c), and the placement of ten implants, five in each hemi-mandible; all **had identical** geometry (9 millimeters (mm) long and 3.5 mm in diameter **(Ø)**) but with two different surface characteristics. Randomization of the interventions was performed using a computer generated block

randomization list (IBM SPSS Statistics® V20 JM.Domenech) that considered the type and position of each implant in the jaw, as well as the type of implant (test and control) placed in each hemimandible.

Both test and control implants were made of **titanium grade 5 following the C1 design** (MIS® Dental Implants, Israel) **with a customized reduced diameter of 3.5 mm with an** internal hexagon connection and with a moderately rough surface **obtained by** sand blasting and acid etching. Without altering **the micro and** macro **characteristic of the** topography, the test implants **received a** mono-molecular layer of multi-phosphonate **(**SurfLink®, **Nano Bridging Molecules, Gland, Switzerland)**. Control implants were identical, but without the monomolecular layer of multi phosphonate. Once the implants were placed, healing abutments of 4.5 mm in diameter were secured and the flaps were closed with absorbable sutures (Vicryl® 4.0, Johnson **&** Johnson, **Sint**-Stevens-Woluwe, Belgium). Sutures **were** removed after 14 days and oral hygiene was maintained for the period of the osseointegration of the implants (three months) (Fig. 1d).

After this period, the induction of peri-implantitis started, by interrupting the oral hygiene regime, and by placing silk ligatures in a subgingival position around the neck of each implant, as previously described by Lindhe [22] (Fig. 2a). Every four weeks for 4 months, all ligatures were replaced to allow for the apical progression of peri-implantitis. After this period, the ligatures were removed (Fig. 2b) and during 4 months, no oral hygiene was provided and dental plaque was allowed to accumulate (spontaneous progression phase) (Fig. 2c). In summary, the experimental phase of this investigation included 8 study visits, the first two belonging to the preparatory phase, four visits where ligatures were monthly placed and replaced (induction phase of the peri-implantitis), and the last two visits without ligatures and oral hygiene (spontaneous progression).

After this period, the animals were euthanized using a lethal dose of sodium Pentothal (40-60 mg/ kg/i.v., Dolethal, Vetoquinol, France) and the mandibles were dissected: half of the specimens **were** processed for non-decalcified histological analysis, and the other half for decalcified soft tissue histological analysis. In this last group the volumetric hard tissue **changes** were analyzed by ***μ*CT** before the histological processing. The histological outcomes of this investigation **have been** presented in an independent report.

*Volumetric hard tissue analysis: **μ*CT** image acquisition and data analysis*

All specimens were scanned before being sectioned using a high resolution multi scale Nano-CT (Skyscan 2211, Bruker microCT NV, Kontich, Belgium) (Fig. 3a). The X-ray source was set at 80 Kv and 90 ***μ***A with a voxel size of 20 micrometers and a 0.5 mm titanium filter. The scanning was performed over a 360º rotation, acquiring images every 0.3º. Once scanned, images were reconstructed using the Feldkamp algorithm [23] and NRecon software (Bruker microCT NV, Kontich, Belgium). The reconstructed images were evaluated with the Data Viewer software (Bruker microCT NV, Kontich, Belgium) and rotated to ensure that the implant was perfectly aligned. **Both the images acquisition and the hard tissue analysis were performed by the same investigator (JSE), following a training of three months in the Department of Biomaterials, at the University of Oslo.** A volume of interest (VOI) of 4 to 6 mm of diameter was selected manually in each specimen from the implant shoulder to first bone to implant contact (VOI) to assess peri-implant bone loss (BL). In this VOI the images were segmented and using global

thresholding methods, the best threshold parameters for bone and for implant were set. Then, by measuring the implant surface free of bone contact the bone loss was calculated. A second VOI, from the first bone to implant contact to the implant apex was selected to assess the total volume of osseointegration. This was done by measuring the intersecting surface between the bone and the implant (BIC) using the method described by Bruker (method note 074, "Osteointegration: analysis of bone around a metal implant" 2015). To perform the analysis, the three-dimensional area comprised between the platform (coronal) and the apex of the implant was considered as the 100% of the volume around implants. Both parameters, bone to implant contact (BIC) and bone loss (BL) were reported as percentage (%) of the total volume around implants and were expressed in cubic millimeters (mm3) (Fig. 3b) (Fig. 3c). Micro-CT data analysis was performed using the CTAn software (Bruker microCT NV, Kontig, Belgium).

Soft tissue contour analysis: STL image and data analysis

Individual trays were fabricated **for** each dog from dental impressions **made** before tooth extractions. Then mandibular impressions using a light/heavy silicon (Elite HD +, Zhermack spa, RO, Italy) were obtained, **1)** after implant placement and before the placement of ligatures (T1), **2)** after **the** ligatures were removed (T2), and **3)** before the sacrifice of the animals (T3) (Fig. 4a) (Fig. 4b) (Fig. 4c). These impressions were poured in dental stone (Fujirock type 4, GC. Corp, Tokyo, Japan) and once the obtained cast models were evaluated to detect possibles imperfections or irregularities of the stone, they were scanned with a desktop 3D scanner (Zfx Evolution Scanner, Zimmer Dental, Bolzano, Italy) to obtain **standard tessellation language (STL) images** (Fig. 4d) (Fig. 4e). These STL files were analyzed by superimposing (matching) the subsequent images using the dedicated software SMOP (Swissmeda Software, Swissmeda AG, Zurich, Switzerland). To obtain a correct matching, three to five **fixed** reference **points** (the anterior teeth and the healing abutments, which were not changed during the investigation) were selected at the baseline and **at** the subsequent follow-up models, then the software performed **automatically** a "rough fit" superimposition. Then, further points of reference **(no less than 10 points)** were selected manually, until **the software achieved** a "fine fit" superimposition **based on a series of mathematical algorithms [24]**. Using the superimposed images, the soft tissue contour linear changes were measured by a **trained** investigator (RDR) using the method previously described by this research group **[25, 26]**. In brief the method consists on drawing a longitudinal buco-lingual slice at the level of each implant obtaining a cross-sectional section that divides each implant in two halves (Fig. 5a). Then, a vertical line was drawn coinciding with the center of the healing abutment and the axis of the implant (Fig. 5b). The soft tissue changes were calculated by measuring the linear changes in both the horizontal (contour) and vertical dimensions (changes in the position of gingival margin (GM)) with an image analysis software (OLYMPUS® cellSens Dimension Desktop 1.14).

To assess **the** contour changes, perpendicular lines were drawn at 1, 2, 3, 4, 5 millimeters (mm) from the GM on the baseline models. These horizontal lines crossed both the lingual and the buccal contours of the crest. Similarly, to assess the vertical changes, vertical lines were drawn from the GM of the three superimposed STL to the top of the healing abutment, at both **the** lingual and buccal aspects. We assigned negative values when the linear contours increased (clinically revealing inflamed soft tissues), while positive values were assigned when the soft tissue contours diminished (clinically revealing **a** loss of

tissue). Similarly, vertical measurements were expressed as positive values when the GM moved apically (clinically revealing a soft tissue dehiscence), while negative values corresponded to a coronal displacement of the position of the GM (clinically revealing inflamed soft tissues).

Three different comparisons were done by subtracting the linear measurements of the models taken at different time points (between T1 and T2 (induction period of peri-implantitis), between T2 and T3 (spontaneous progression of peri-implantitis), and between T1 and T3 (changes throughout the investigation) (Fig. 5c).

Data Analysis

The dog was considered as the unit of all the analysis. The hard tissue volumetric analysis (**μCT**) was evaluated at the end of the investigation after retrieving the specimens before their decalcification. **μCT** data were expressed as means, standard deviation (SD) and confidence intervals. The soft tissue contour linear analysis were calculated at three different time points (T1, T2, T3) and data were also expressed as means, standard deviation (SD) and confidence intervals. Shapiro-Wilk normality tests were performed to assess the data distribution. T-tests were used for the inter-group comparisons, while ANOVA tests were used to compare the differences of the contour linear measurements in terms of height (vertical) and width (horizontal). Bonferroni corrections were performed for multiple comparisons. The alpha error was set at 0.05.

Results

Healing after all the surgical procedures was uneventfully in all the experimental animals, and no implants were lost during the study. **Soft and hard tissue analysis were performed in all the dogs, but not in all the implants. A**mong the 80 implants placed, the most mesially placed in each hemi-mandible were not included in the analysis, since in these implants a dehiscence defect was surgically to test a hypothesis not studied in the present investigation. From the remaining 64 implants, **μCT** volumetric analysis was carried out **for** 32 implants (16 test and 16 control) and soft tissue contour linear changes **for** 47 implants (24 test and 23 control). Seventeen implants (8 test and 9 control) were excluded due to the presence of imperfections in the cast models, which made impossible a correct matching of the STL files. The soft tissue analysis resulted in 36 measurements per-each combination of the three **superimposed** STLs, which corresponded to a total of 1692 linear measurements **for the 47 implants considered for the soft tissue analysis**, 1410 being horizontal (705 buccal and 705 lingual) and 282 verticals (141 buccal and 141 lingual).

*Hard tissue **μCT** analysis*

Detailed description of the **μCT** data is depicted in Table 1. Test implants lost more bone than control implants at the end of the experimental peri-implantitis (42.94 mm3 vs 37.86 mm3, respectively), although these differences were not statistically significant (p=0.165). The percentage of total BIC was similar between both implant groups, being slightly higher in the control group when compared with the test group

(53.98 mm3 vs 49.82 mm3, respectively) (p=0.132). Both implants lost around 40% of their peri-implant bone support due to peri-implantitis **(Supplementary graph 1)**.

Soft tissue linear measurements

Horizontal measurements were drawn at 1, 2, 3, 4 and 5 mm from the GM of the baseline models (T1), thus obtaining 10 measurements (5 buccal and 5 lingual) for each implant, with a total of 30 measurements for the three time points of the study (induction, spontaneous progression and begin-end). Table 2 depicts the inter-group comparisons of these horizontal buccal measurements. Differences between test and control implants were not statistically significant, irrespective of the measurement level and the healing periods. During the induction period, positive values were recorded for both groups in the most coronal levels (1 and 2 mm) of the buccal soft tissue contour, revealing a loss of soft tissue, whereas negative values were observed more apically at 3, 4 and 5 mm, revealing an increase in soft tissue contour. During the progression period, once ligatures were removed, the loss of soft tissue contours continued, irrespective of the groups. When assessing the period between baseline **and** the end of the study, soft tissue contour increase was only observed at 3 mm for test groups, whereas a similar increase was noted in control groups at the middle and apical levels (3, 4 and 5 mm) **(Graph 1).**

Table 3 depicts the inter-group comparisons of the horizontal lingual measurements. Similar to the buccal changes, differences between test and control implants were not statistically significant. During the induction period, increase in soft tissue contours was observed in both groups, revealing an inflamed lingual soft tissue, except in the coronal aspect (1 mm for test group and 1 and 2 mm for control group, respectively) where loss of contour was noted. During the spontaneous progression period, loss of soft tissue contour occurred in both groups. When comparing baseline with the end of the study, the lingual soft tissue contour also diminished, irrespective of the groups, although a higher loss was noted in the lingual soft tissue contour in the control group **(Graph 2).**

Table 4 depicts the inter-group comparisons of the vertical soft tissue linear measurements at both the buccal and lingual mucosal margins during the different study intervals. Differences between groups were not significant, neither at buccal nor at lingual aspects. Throughout the study the position of soft tissue margin moved apically, except the time point just after the progression period where a coronal displacement of buccal soft tissue margin was observed for both groups. While test implants presented a higher incidence of buccal soft tissue dehiscence after the induction period and between the baseline and the end of the study compared to control implants, lingual soft tissue dehiscence was more pronounced in the control group at every time point. **When comparing changes in vertical soft tissue contour between the buccal and lingual aspect, it was observed that during the induction period, the buccal soft tissue contour receded 0.95 mm in comparison with the lingual contour in test and 0.66 mm in control implants, respectively. These differences between buccal and lingual contour reduction were statistically significant (p=0.004). During the progression phase more vertical reduction of the soft tissue contours was observed at the lingual aspect compared with the buccal aspect, in both test (0.39 mm) and control implants (0.66 mm). Differences between buccal and lingual contour reduction were statistically significant only in the control group (p=0.004). When considering vertical contour modification on**

the complete experimental period (Baseline-End), the buccal soft tissue contour was reduced 0.55 mm more than the lingual contour only in the test group (p=0.003), while in the control group, both lingual and buccal had similar amount of contour reduction (Graph 3).

Discussion

This pre-clinical in vivo investigation has used a novel technology to assess the hard and soft tissue changes occurring during and after experimental peri-implantitis with the goal of comparing two implants with identical micro and macro topographical design, but having the tested implants a unique bioactive external surface layer of multi-phosphonate molecules, which has shown an increased osseointegration velocity in preclinical studies. The study hypothesis was based in the assumption that a more rapid osseointegration would implicate a harder resistance to de-osseointegration, when implants were exposed to a well validated ligature induced peri-implantitis model. **The** de-osseointegrated dynamics were evaluated at two levels, first by assessing the net 360º bone loss and bone to implant contact using ***μ*CT** scans, secondly by measuring the changes **of** the soft tissue linear contours, both buccally and lingually resulting from the induction and progression phases of ligature induced peri-implantitis. The ***μ*CT** data indicated that a pronounced bone loss occurred after peri-implantitis induction and progression **for** both test and control implants. Even though more bone loss occurred **for** the test implants, these differences were not statistically significant when compared with the control implants. The results on the soft tissue linear changes showed that during the induction period there was a horizontal increase of soft tissue contours, while during the spontaneous progression, these soft tissue contours receded. Again, differences between test and control implants were not statistically significant, at both the buccal and lingual sides. In vertical dimension, a soft tissue dehiscence was observed for both groups, being more pronounced at the buccal aspect after the induction period. No significant differences were observed between **the** test and control implants.

The increase in soft tissue contour reported in both implant surfaces during the induction period may be explained, mainly by the severe inflammatory reaction resulting from the placement of the ligatures. Additionally, the submarginal placement of the ligatures may also increase the marginal tissue contours. Durign the progression phase, the reported reduction in tissue contours in both test and control implants may be explained by; first, the removal of the ligatures, which reduced the marginal contours, and secondly, by the resulting tissue loss as a consequence of the inflammation decrease and the net tissue loss, what resulted in a marked recession.

The differential microscopic design of the tested implants was characterized by a hydrophilic surface **that consisted of** a monomolecular layer of multi phosphonates covalently bound to the implant surface. This implant surface was previously tested in a preclinical investigation in sheep, evaluating as outcome the quality of osseointegration using histology, histomorphometry and scanning electron microscopy [19]. The results from **that** investigation showed higher removal torque values and higher BIC percentages in implants with this new hydrophilic surface compared with similar implants without it, mainly during the first 8 weeks after implant placement, although higher BIC values were maintained up to 52 weeks. These results prompted the authors to define this novel implant surface as osteoconductive, since more woven bone matrix was observed on the tested implant surface, especially during the early healing time [19]. Since

in the present investigation the 360º BIC values were calculated once the peri-implantitis was induced, we cannot verify or refute this potential osteoinductivity, nor the increased pace of osseointegration, but clearly shows that once osseointegrated, these implants were similarly subject to de-osseointegration after peri-implantitis induction, when compared with implants of identical design, but without the hydrophilic surface. In another in vivo investigation using a similar peri-implantitis model ant testing two implant surfaces characterised by silver electrodeposition and 3-(triethoxysilyl) propyl succinic anhydride (TEPSA) silane, compared to non-modified implants, the ***μ*CT** results also did not report significant differences between groups, even though significant differences were described at **the** histological level **[27]**.

Implants with the multiphosphonate surface layer were tested also in a randomized clinical trial (RCT) in humans, in both **the** mandibles and maxilla [20]. No implant failures and no differences in peri-implant mucosal inflammation were observed in both test and control implants during the entire follow-up (12 months). However, after 3 and 12 months from implant loading, less marginal bone loss (MBL) was observed for implants with the treated surface when compared to those without it, although differences were not statistically significant. This study showed that the tested surface was safe but did not provide any significant added benefit over control implants. The authors stated that more studies **under** more critical conditions were needed [20]. The results from this clinical investigation were confirmed with the results from the present study, since the tested surface did not provide an added value in the prevention of the hard tissue changes associated with peri-implantitis.

In the present study we have utilized a novel approach to measure the soft tissue contour changes associated with the development of peri-implantitis. The obtained results **are in line** with the ***μ*CT** data since no significant differences were found between **the** test and control implants. During the active induction period of peri-implantitis predominated the inflammatory component, characterized by a horizontal increase in the contour both at lingual and buccal sides, irrespective of the groups. During this period, soft tissue dehiscence defects developed mainly buccally, what may be explained by the ligatures, whose knots were predominantly tied buccally. Similarly, there were no significant differences between the tested implants and the controls.

During the disease spontaneous progression of peri-implantitis, a decrease in the horizontal soft tissue contour was observed at both buccal and lingual aspects, irrespective of the groups. Similarly, further loss in vertical dimension was noted in both groups, what can be explained by the partial resolution of the inflammation resulting from the removal of the ligatures, what translated in a net hard and soft tissue loss. Finally, when comparing soft tissue contour changes from the start to the end of the investigation, there was a clear horizontal loss in soft tissue contours at all heights in the lingual aspect, irrespective of the groups. However, a slight increase was noted at midlevel (3 mm) in the test group and at both middle and apical levels in the control group (3 mm, 4 mm, 5 mm), **that** confirms that the pronounced bone loss identified by ***μ*CT** did not fully translate into loss of soft tissue contours. The results obtained in this investigation in terms of soft tissue inflammation and bone loss during ligature induced peri-implantitis are similar to those reported in other preclinical studies using the same experimental model **[28 - 32]**, although the main difference with these studies is the novel methodology used in this investigation to assess the bone volumetric and soft tissue contour changes.

The use of Micro-Ct to measure peri-implant bone changes has shown to be a useful and precise tool to quantify bone density and to measure its volume and microarchitecture, particularly at the trabecular level **[33, 34]**. Even though most of the investigations have used Micro-Ct to assess bone volume changes after regenerative procedures **[26, 35, 36]**, it has also been utilized to assess bone changes in peri-implantitis, in both preclinical and clinical investigations **[27, 33, 37]**. These studies, similar to the present investigation reported an altered morphology of the peri-implant bone and a significant three-dimensional bone to implant contact (BIC) loss after ligature induced peri-implantitis.

The use of optical scanners to superimpose and analyze virtual stereolithographic (STL) models, have also been used reliably to evaluate the soft tissue volumetric and contour linear changes, in both preclinical and clinical investigations **[25, 26, 38 - 41]**. The present investigation, however, is the first time to use this technology to assess these changes in experimental peri-implantitis. Recently a similar methodology was used in a human study to assess the volumetric changes after a bone regenerative intervention of peri-implantitis lesions **[42]**. In this study STL files were obtained before **the** regenerative procedure, and after 1 and 6 months. The results showed that peri-implant soft tissues underwent significant volumetric changes during all the different time points, especially at the marginal region.

As any pre-clinical in vivo study, this investigation has important limitations in regard to its possible translation of the obtained results to patients. Similarly, the limited sample size as a consequence to the need of reducing the number of experimental animals, may limit the validity of the results. **The experimental periimplantitis model used in this investigation using submarginally placed ligatures, exerts a mechanical effect superimposed to the chronic inflammation resulting from biofilm accumulation and this effect will not occur in the naturally developed disease. Furthermore, some methodological limitations related to the used method to analyse both the soft and hard tissue contours need to be mentioned. For an accurate record of the soft tissue profiles, the impressions need to be very precise and accurate, containing all the anatomical details. Hence, not all implants were analysed in this investigation, but only those with precise recordings. However, with this selected material, the use of the presented method demonstrated a high degree of precision, with a measurement error below 20 μm, and an excellent reproducibility with coefficients of variation ranging from 0.05 to 0.5% [43, 44]. The μCT analysis method used has the main limitation that soft tissue changes are not assessed, what implies the use of different methodological tools to assess the hard and the soft tissues.** Finally, the lack of histological results in this report may limit a true understanding of the tissue behavior during the different phases of peri-implantitis.

Conclusion

Within these limitations of this pre-clinical investigation, the results of this study evaluating the hard volumetric and soft tissue contour changes, did not confirm that implants **treated** with a monolayer of multi-phosphonate molecules provided a more resistant environment to the pathological changes occurring in ligature induced peri-implantitis. Therefore, future investigations are needed to confirm whether the addition to this implant surface provides a significant additional value to long-standing dental implant osseointegration.

Compliance with Ethical Standards

Conflict of Interest:

The authors (Riccardo Di Raimondo, Javier Sanz-Esporrin, Ignacio Sanz-Martín, Fabio Vignoletti, Javier Nuñez, Fernando Muñoz, Håvard Jostein Haugen and Mariano Sanz) declare no conflict of interest in relation with the results of this study and the ensuing publication

Funding: This study was partially supported with a research contract between the University Complutense of Madrid and MIS Dental Implants

Ethical approval: AE-LU-001/04/16

Informed consent: For this type of study, formal consent is not required.

References

1. Moraschini V, da C. Poubel LA, Ferreira VF, dos SP. Barboza E (2015) Evaluation of survival and success rates of dental implants reported in longitudinal studies with a follow-up period of at least 10 years: a systematic review. Int. J. Oral Maxillofac. Surg.; 44: 377–388

2. Pjetursson BE, Asgeirsson AG, Zwahlen M, Salier I (2014) Improvements in implant dentistry over the last decade: comparison of survival and complication rates in older and newer publications. Int J Oral Maxillofacial Implants; 29 (suppl): 308-324

3. Caton J, Armitage G, Berglundh T, et al. (2018) A new classification scheme for periodontal and peri-implant diseases and conditions – Introduction and key changes from the 1999 classification. J Clin Periodontol.;45(Suppl 20): S1–S8.

4. Berglundh T, Armitage G, et al. (2018) Peri- implant diseases and conditions: Consensus report of workgroup 4 of the 2017 World Workshop on the Classification of Periodontal and Peri- Implant Diseases and Conditions. J Clin Periodontol.;45(Suppl 20): S286–S291.

5. Schwarz F, Derks J, Monje A, Wang HL (2018) Peri- implantitis. J Clin Periodontol.;45(Suppl 20): S246–S266.

6. Derks J & Tomasi C. Peri-implant health and disease (2015) A systematic review of current epidemiology. J Clin Periodontol; 42 (Suppl. 16): S158–S171.

7. Krebs M, Kesar N, Begic A, von Krockow N, Nentwig G-H, Weigl P (2019) Incidence and prevalence of peri-implantitis and peri-implant mucositis 17 to 23 (18.9) years postimplant placement. Clin Implant Dent Relat Res.;1–8.

8. Renvert S, Persson GR, Pirih FQ, Camargo PM (2018) Peri- implant health, peri- implant mucositis, and peri- implantitis: Case definitions and diagnostic considerations. J Clin Periodontol.;45(Suppl 20): S278–S285.

9. Ivanosky S & Lee R (2017) Comparison of peri-implant and periodontal marginal soft tissues in health and disease. Periodontology 2000; 0: 1-15

10. Giovannoli JL, Roccuzzo M, Albouy JP, Duffau F, Lin GH, Serino G (2019) Local risk indicators - Consensus report of working group 2. Intern Dent Journal; 69 (Suppl): 7-11

11. Dreyer H, Grischke J, Tiede C, et al. (2018) Epidemiology and risk factors of peri-implantitis: A systematic review. J Periodont Res.; 00:1-25.

12. Saulaci N & Schaller B (2019) Prevalence of Peri-Implantitis in Implants with Turned and Rough Surfaces: a Systematic Review. J Oral Maxillofac Res; 10(e1): 1-12

13. Jordana F, Susbielles L, Colat-Parros J (2018) Periimplantitis and implant body roughness: a systematic review of literature. Implant Dentistry; 27 (6): 672-681

14. Asensio G, Vazquez-Lasa B, Rojo L (2019) Achievements in the Topographic Design of Commercial Titanium Dental Implants: Towards Anti-Peri-Implantitis Surfaces. J. Clin. Med.; 8 (11): 1982-2000

15. Jansen JA, Brugge P, Van Der Waal E, Vredenberg A, Wolke J (2003). Osteocapacities of calcium phosphate ceramics. In: Ellingsen JE, Lyngstadaas SP (eds). Bioimplant Inter- face. Boca Raton, FL: CRC:305-22.

16. Junker R, Dimakis A, Thoneick M, Jansen JA (2009). Effects of implant surface coatings and composition on bone integration: a systematic review. Clin. Oral Impl. Res.; 20 (Suppl. 4):185–206

17. Viornery C, Chevolot Y, Léonard D, Aronsson BO, Péchy P, Mathieu HJ, Descouts P, Grätzel M (2002). Surface modification of titanium with phosphonic acid to improve bone bonding: Characterization by XPS and ToF-SIMS. Langmuir; 18, 2582–2589.

18. Viornery C, Guenther HL, Aronsson BO, Péchy P, Descouts P, Grätzel M (2002). Osteoblast culture on polished titanium disks modified with phosphonic acids. J. Biomed. Mater. Res.; 62, 149–155.

19. Von Salis-Soglio M, Stübinger S, Sidler M, Klein K, Ferguson S, Kämpf K, et al. (2014). A Novel Multi-Phosphonate Surface Treatment of Titanium Dental Implants: A Study in Sheep. J. Funct. Biomater; 5 (3): 135–57.

20. Esposito M, Dojcinovic I, Germon L, Lévy N, Curno R, Buchini S, Péchy P, Aronsson BO (2013). Safety and efficacy of a biomimetic monolayer of permanently bound multi-phosphonic acid molecules on dental implants: 1year post-loading results from a pilot quadruple-blinded randomised controlled trial. Eur. J. Oral Implantol.; 6: 227–236.

21. Vignoletti F. & Abrahamsson I (2012) Quality of reporting of experimental research in implant dentistry. Critical aspects in design, outcome assessment and model validation. Journal of Clinical Periodontology 39(Suppl. 12): 6–27

22. Lindhe J, Berglundh T, Ericsson I, Liljenberg B, Marinello C (1992) Experimental breakdown of peri-implant and periodontal tissues. A study in the beagle dog. Clin Oral Implants Res.;3(1):9-16.

23. Feldkamp LA, Davis LC, Kress JW (1984) Practical cone-beam algorithm. J Opt Soc Am; 1 (6): 612-619

24. Becker K, Wilmes B, Grandjean C, Drescher D (2018) Impact of manual control point selection accuracy on automated surface matching of digital dental models. Clin Oral Investig.; 22 (2): 801-810

25. Di Raimondo R, Sanz-Esporrin J, Pla R, Sanz-Martin I, Luengo F, Vignoletti F, Nuñez J, Sanz M (2020) Alveolar crests contour changes after guided bone regeneration using different biomaterials: an experimental in vivo investigation. Clin Oral Invest; 24 (7): 2351-2361

26. Di Raimondo R, Sanz-Esporrin J, Sanz-Martin I, Pla R, Luengo F, Vignoletti F, Nuñez J, Sanz M (2020) Hard and soft tissue changes after guided bone regeneration using two different barrier membranes: an experimental in vivo investigation. Clin Oral Invest; https://doi.org/10.1007/s00784-020-03537-5

27. Godoy-Gallardo M, Manzanares-Cespedes MC, Sevilla P, Nart J, Manzanares N, Manera JM., Gil FJ, Body SK, Rodriguez D (2016) Evaluation of bone loss in antibacterial coated dental implants: An experimental study in dogs. Mater Sci Eng C Mater Biol Appl. 1; 69: 538-45.

28. Berglundh, T, Gotfredsen, K, Zitzmann, NU, Lang, NP, Lindhe, J (2007) Spontaneous progression of ligature induced peri-implantitis at implants with different surface roughness: an experimental study in dogs. Clin. Oral Impl. Res. 18; 655–661

29. Albouy JP, Abrahamsson I, Persson LG, Berglundh T (2008) Spontaneous progression of peri-implantitis at different types of implants. An experimental study in dogs. I: clinical and radiographic observations. Clin Oral Implants Res.; 19 (10): 997- 1002.

30. Albouy JP, Abrahamsson I, Persson LG, Berglundh T (2009) Spontaneous progression of ligatured induced peri-implantitis at implants with different surface characteristics. An experimental study in dogs II: histological observations. Clin Oral Implants Res.; 20 (4): 366-71.

31. Fickl S, Kebschull M, Calvo-Guirado JL, Hurzeler M, Zuhr O (2015) Experimental Peri-Implantitis around Different Types of Implants – A Clinical and Radiographic Study in Dogs. Clin Implant Dent Relat Res.;17 Suppl 2: e661-9

32. Sanz-Esporrin J, Blanco J, Sanz-Casado JV, Muñoz F, Sanz M (2019) The adjunctive effect of rhBMP-2 on the regeneration of peri-implant bone defects after experimental peri-implantitis. Clin Oral Impl Res; 30 (12): 1209-1219

33. Maglione M, Vaccari L, Mancini L, Ciancio R, Bedolla DE, Bevilacqua L, Tonellato P (2019) Micro-ATR FTIR, SEM-EDS and X-ray micro-CT: an innovative multi-technique approach to investigate bone affected by peri-implantitis. Int J Oral Maxillofac Implants; 34 (3): 631–641

34. Finelle G, Papadimitriou DEV, Souza AB, Katebi N, Gallucci GO, Araujo MG (2015) Peri-implant soft tissue and marginal bone adaptation on implant with non-matching healing abutments: micro-CT analysis. Clin. Oral Impl. Res.; 26: e42–e46

35. Thoma DS, Jung U-W, Park J-Y, Bienz SP, Husler J, Jung RE (2017). Bone augmentation at peri-implant dehiscence defects comparing a synthetic polyethylene glycol hydrogel matrix versus standard guided bone regeneration techniques. Clin. Oral Impl. Res. 28: e76–e83.

36. Khobragade P, Jain A, Setlur Nagesh SV, Andreana S, Dziak R, Sunkara SK, Ionita CN (2015). Micro-Computed tomography (CT) based assessment of dental regenerative therapy in the canine mandible model. Proceedings of SPIE-the International Society for Optical Engineering, 17, 9417

37. Qian W, Qiu J, Liu X (2019) Minocycline Hydrochloride Loaded Graphene Oxide Films On Implant Abutments For Peri-Implantitis Treatment In Beagle Dogs. J Periodontol., doi: 10.1002/JPER.19-0285

38. Sanz-Martin I, Benic GI, Hammerle CH, Thoma, DS (2016) Prospective randomized controlled clinical study comparing two dental implant types: volumetric soft tissue changes at 1 year of loading. Clin Oral Implants Res; 27: 406-411

39. Sanz-Martin I, Vignoletti F, Nuñez J, Permuy M, Muñoz F, Sanz-Esporrin J, Fierravanti L, Shapira L, Sanz M (2017) Hard and soft tissue integration of immediate and delayed implants with a modified coronal macro design: Histological, micro CT and volumetric soft tissue changes from a pre-clinical in vivo study. Journal of Clinical Periodontology 44 (8): 842-853

40. Sanz-Martin I, Ferrantino L, Vignoletti F, Nunez J, Baldini N, Duvina M, Alcaraz J, Sanz M (2018) Contour changes after guided bone regeneration of large non-contained mandibular buccal bone defects using deproteinized bovine bone mineral and a porcine-derived collagen membrane: an experimental in vivo investigation. Clin Oral Invest; 22(3): 1273-1283

41. Basler T, Naenni N, Schneider D, Hammerle CHF, Jung R, Thoma DS (2018) Randomized controlled clinical study assessing two membranes for guided bone regeneration of peri-implant bone defects: 3-year results. Clin Oral Implants Res.; 29 (5): 499-507

42. Galarraga-Vinueza ME, Obreja K, Magini R, Sculean A, Sader R, Schwarz F (2020). Volumetric assessment of tissue changes following combined surgical therapy of peri-implantitis: A pilot study. J Clin Periodontol.;00:1–10. https://doi.org/10.1111/jcpe.13335

43. Mehl A, Gloger W, Kunzelmann KH, Hickel R (1997) A new optical 3-D device for the detection of wear. J Dent Res.;76 (11): 1799-807

44. Windisch SI, Jung, RE, Sailer I, Studer SP, Ender A, Hämmerle CHF (2007) A new optical method to evaluate three-dimensional volume changes of alveolar contours: a methodological in vitro study. Clin Oral Implants Res.; 18: 545-551

Figure legends

Fig. 1. Images of the experimental surgeries. Preparatory phase. (a) Hemi-section of P2, P3, P4 and M1. (b) Extractions of P2, P3, P4 and M1. (c) Healed ridge crest after 3 months from the extractions. (d) Implant placement before inducted peri-implantitis.

Fig. 2. Images of the experimental peri-implantitis model. (a) Placement of ligatures around each implant. (b) peri-implant tissues after the induction of peri-implantitis with subgingival ligatures. (c) peri-implant tissues after the spontaneous progression of peri-implantitis once the ligatures were removed.

Fig. 3. Images of the hard tissue analysis with Micro-Ct. (a) Implant section before the analysis. (b) Cross-sectional section that divides the implant into two equal parts. Blue area represents implant surface that has lost bone support. Yellow area represents tridimensional bone to implant contact. (c) Tridimensional implant section used for the analysis; Blue area represents tridimensional bone loss (BL), while yellow area represents tridimensional bone to implant contact (BIC).

Fig. 4. Images of the impressions and Stereolithography (STL) image acquisition. (a) Individual impression trays of each dog and impression materials. (b) Placement of both light and heavy putty silicon inside the individual tray. (c) Impression of the mandible. (d) Cast model during scan process with the extraoral scanner. (e) Acquisition of the STL file.

Fig. 5. Images of the soft tissue analysis. (a) STL files matching of the entire mandible at the three different time points of the study. Red line corresponds to the cross-sectional section that divides the implant into two equal parts. (b) STL files matching at implant level. (c) Vertical and horizontal linear measurements between the different time points of the study at both lingual and buccal aspects. Yellow line represents pre ligatures contour (Baseline); Green line represents post-ligatures contour; Grey line represents post-spontaneous progression contour.

Tables

Table 1. Inter-group comparisons (mean difference (SD) (95% CI)) in the Micro-CT bone volumetric measurements assessing bone to implant contact (BIC) and bone loss (BL).

Table 2. Inter-group comparisons of the horizontal buccal contour changes of test and control groups between the different time points of the study (induction, progression, and begin-end) at five different crestal levels (1mm, 2 mm, 3 mm, 4 mm, 5mm). (mean difference (95% CI)).

Table 3. Inter-group comparisons of the horizontal lingual contour changes of test and control groups between the different time points of the study (induction, progression, and begin-end) at five different crestal levels (1mm, 2 mm, 3 mm, 4 mm, 5mm). (mean difference (95% CI)).

Table 4. Vertical Buccal and lingual contour changes of test and control groups between the different moments of the study (induction, progression, and begin-end). Inter-group comparisons (mean difference (95% CI)). Bucco-lingual comparisons.

Graphs

Graph 1. Graphic representation of the horizontal buccal contour changes of soft tissue between the different time points of the study (induction, progression, and begin-end) at five different crestal levels (1mm, 2 mm, 3 mm, 4 mm, 5mm). Blue and green rectangles corresponded to test and control group, respectively.

Graph 2. Graphic representation of the horizontal lingual contour changes of soft tissue between the different time points of the study (induction, progression, and begin-end) at five different crestal levels (1mm, 2 mm, 3 mm, 4 mm, 5mm). Blue and green rectangles corresponded to test and control group, respectively.

Graph 3. Graphic representation of the vertical buccal and lingual contour changes of test and control groups between the different moments of the study (induction, progression, and begin-end. Blue and green rectangles corresponded to test and control group, respectively.

Supplementary graph

Supplementary graph 1. Graphic representation of the hard tissue volumetric analysis obtained by means of µCT. Blue and green rectangles corresponded to test and control group, respectively.

Tables

Table 1. Micro-Ct volumetric data of hard tissue. All measurements are expressed in cubic millimetres

Measurements	Mean (SD) C Group	IC 95% (Inf. Lim; Sup. Lim)	Mean (SD) T Group	IC 95% (Inf. Lim; Sup. Lim)	Mean Δ C/T	IC 95% (Inf. Lim; Sup. Lim)	p
% BIC 360	53.98 (5.21)	49.62; 58.35	49.82 (4.64)	45.53; 54.12	4,15	-1.38; 9.70	0,132
% Bone Loss	37.86 (6.48)	32.44; 43.28	42.94 (3.72)	39.49; 46.38	-5,07	-11.09; 0.94	0,165

SD:standard deviation; IC 95%: confidence interval 95%; Δ: mean difference; T: test group; C: control group; BIC: bone to implant contact

Table 2. Prophilometric linear changes of soft tissue at buccal aspect. Inter-group comparisons. All measurements are expressed in millimetres

Period	Measurements	Mean (SD) T Group	IC 95% (Inf. Lim; Sup. Lim)	Mean (SD) C Group	IC 95% (Inf. Lim; Sup. Lim)	Mean Δ T/C	IC 95% (Inf. Lim; Sup. Lim)	p
Induction	1 mm	0.58 (0.64)	0.04; 1.13	0.65 (0.49)	0.24; 1.06	-0,07	-0.68; 0.54	0,793
	2 mm	0.04 (0.61)	-0.47; 0.55	0.32 (0.64)	-0.20; 0.86	-0,28	-0.96; 0.38	0,294
	3 mm	-0.21 (0.66)	-0.77; 0.33	-0.08 (0.67)	-0.65; 0.47	-0,12	-0.84; 0.58	0,674
	4 mm	-0.24 (0.84)	-0.95; 0.46	-0.30 (0.74)	-0.92; 0.32	0,05	-0.80; 0.90	1,000
	5 mm	-0.42 (1.07)	-1.45; 0.68	-0.61 (0.92)	-1.38; 0.16	0,18	-0.89; 1.25	0,753
Progression	1 mm	-0.05 (0.47)	-0.45; 0.34	0.09 (0.45)	-0.27; 0.47	-0,15	-0.65; 0.34	0,401
	2 mm	0.05 (0.59)	-0.44; 0.55	-0.28 (0.51)	-0.71; 0.14	0,33	-0.25; 0.93	0,345
	3 mm	0.29 (0.55)	-0.16; 0.75	-0.05 (0.37)	-0.36; 0.25	0,34	-0.15; 0.84	0,115
	4 mm	0.35 (0.35)	0.06; 0.65	0.06 (0.40)	-0.27; 0.40	0,28	-0.12; 0.69	0,248
	5 mm	0.46 (0.29)	0.19; 0.73	0.41 (0.67)	-0.14; 0.98	0,04	-0.55; 0.63	0,355
Begin-End	1 mm	0.41 (0.37)	0.10; 0.73	0.59 (0.70)	0.009; 1.18	-0,18	-0.78; 0.42	0,294
	2 mm	0.11 (0.41)	-0.23; 0.46	0.07 (0.71)	-0.52; 0.66	0,04	-0.58; 0.67	0,916
	3 mm	-0.06 (0.48)	-0.46; 0.34	-0.12 (0.50)	-0.54; 0.30	0,06	-0.46; 0.59	0,793
	4 mm	0.005 (0.90)	-0.75; 0.76	-0.16 (0.41)	-0.51; 0.18	0,17	-0.58; 0.92	0,753
	5 mm	0.13 (1.07)	-0.86; 1.12	-0.19 (0.41)	-0.54; 0.14	0,33	-0.55; 1.21	0,817

SD:standard deviation; IC 95%: confidence interval 95%; Δ: mean difference; T: test group; C: control group.

Table 3. Prophilometric linear changes of soft tissue at lingual aspect. Inter-group comparisons. All measurements are expressed in millimetres

Period	Measurements	Mean (SD) T Group	IC 95% (Inf. Lim; Sup. Lim)	Mean (SD) C Group	IC 95% (Inf. Lim; Sup. Lim)	Mean Δ T/C	IC 95% (Inf. Lim; Sup. Lim)	p
Induction	1 mm	0.49 (0.70)	-0.53; 0.63	0.13 (0.87)	-0.59; 0.86	-0,08	-0.93; 0.76	0,674
	2 mm	-0.25 (0.44)	-0.63; 0.11	0.05 (0.69)	-0.52; 0.64	-0,31	-0.94; 0.30	0,462
	3 mm	-0.44 (0.44)	-0.81; -0.06	-0.14 (0.44)	-0.51; 0.22	-0,29	-0.77; 0.18	0,115
	4 mm	-0.41 (0.45)	-0.79; -0.02	-0.14 (0.39)	-0.46; 0.18	-0,26	-0.72; 0.18	0,115
	5 mm	-0.38 (0.42)	-0.74; -0.03	-0.09 (0.41)	-0.43; 0.25	-0,29	-0.74; 0.15	0,115
Progression	1 mm	0.30 (0.57)	-0.17; 0.79	0.47 (0.42)	0.11; 0.83	-0,16	-0.71; 0.38	0,529
	2 mm	0.52 (0.48)	0.12; 0.92	0.52 (0.40)	0.18; 0.85	0,003	-0.47; 0.47	0,875
	3 mm	0.48 (0.45)	0.10; 0.87	0.38 (0.28)	0.14; 0.62	0,09	-0.30; 0.50	0,401
	4 mm	0.39 (0.53)	-0.04; 0.84	0.26 (0.26)	0.04; 0.48	0,13	-0.31; 0.58	0,529
	5 mm	0.39 (0.53)	-0.04; 0.84	0.17 (0.29)	-0.06; 0.42	0,22	-0.23; 0.68	0,248
Begin-End	1 mm	0.35 (0.50)	-0.06; 0.77	0.60 (0.87)	-0.12; 1.34	-0,25	-1.02; 0.51	0,401
	2 mm	0.26 (0.51)	-0.16; 0.69	0.51 (0.79)	-0.15; 1.17	-0,24	-0.95; 0.46	0,674
	3 mm	0.04 (0.45)	-0.33; 0.42	0.24 (0.54)	-0.21; 0.69	-0,19	-0.73; 0.34	0,563
	4 mm	0.01 (0.39)	-0.31; 0.35	0.12 (0.55)	-0.34; 0.59	-0,10	-0.62; 0.41	0,916
	5 mm	0.01 (0.36)	-0.29; 0.32	0.08 (0.62)	-0.43; 0.60	-0,07	-0.61; 0.47	0,529

SD:standard deviation; IC 95%: confidence interval 95%; Δ: mean difference; T: test group; C: control group.

Table 4. Vertical linear changes of soft tissue at both buccal and lingual aspect. Inter-group comparisons. All measurements are expressed in millimetres

Period	Measurements THA- GM	Mean Δ (SD) T Group	IC 95% (Inf. Lim; Sup. Lim)	Mean Δ (SD) C Group	IC 95% (Inf. Lim; Sup. Lim)	Mean Δ T/C	IC 95% (Inf. Lim; Sup. Lim)	p
Induction	**Buccal**	1.26 (0.47)	0.86; 1.65	1.12 (0.60)	0.62; 1.63	0,13	-0.45; 0.71	0,834
	Lingual	0.31 (0.89)	-0.43; 1.06	0.46 (0.65)	-0.07; 1.01	-0,15	-0.99; 0.68	0,753
Mean Δ Buccal-Lingual (p value)		0.95 (0.004) ***		0.66 (0.004) ***				
Progression	**Buccal**	-0.33 (0.42)	-0.69; 0.01	-0.33 (0.38)	-0.66; -0.01	-0,004	-0.44; 0.43	0,958
	Lingual	0.05 (0.45)	-0.32; 0.43	0.33 (0.43)	-0.02; 0.69	-0,28	-0.75; 0.19	0,208
Mean Δ Buccal-Lingual (p value)		-0.39 (0.078)		-0.66 (0.004) ***				
Begin-End	**Buccal**	0.92 (0.61)	0.41; 1.43	0.79 (0.64)	0.25; 1.33	0,12	-0.54; 0.80	0,916
	Lingual	0.36 (0.50)	-0.06; 0.78	0.80 (0.69)	0.22; 1.38	-0,43	-1.09; 0.21	0,227
Mean Δ Buccal-Lingual (p value)		0.55 (0.003) ***		-0.008 (0.930)				

SD:standard deviation; IC 95%: confidence interval 95%; THA-GM: top of healing abutment (THA) and gingival margin (GM) Δ: mean difference; T: test group; C: control group. *: statistical significant differences (p<0.05)**

Figure 1

Click here to access/download;Figure;Fig 1.tif

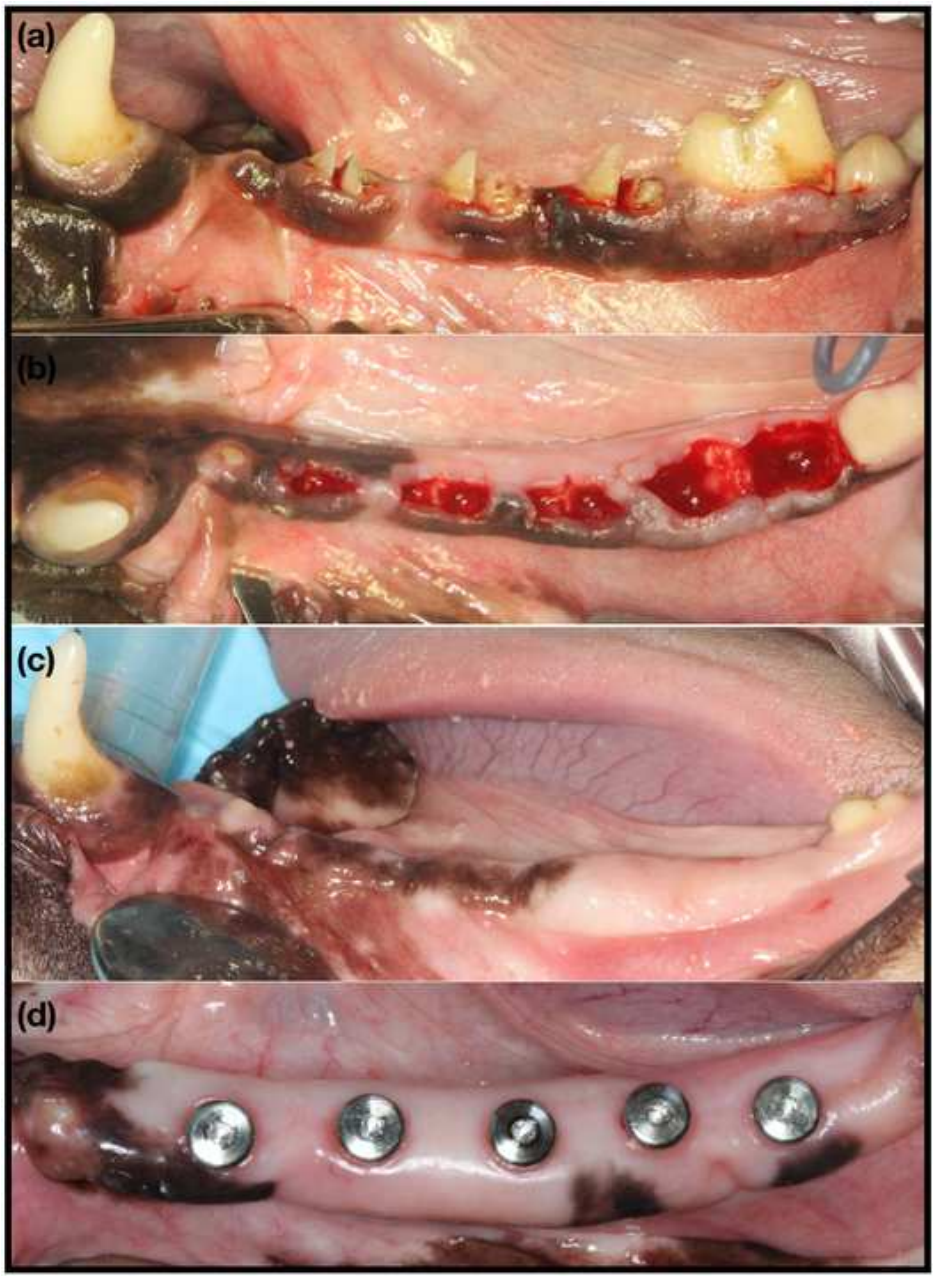


Figure 2

Click here to access/download;Figure;Fig 2.tif

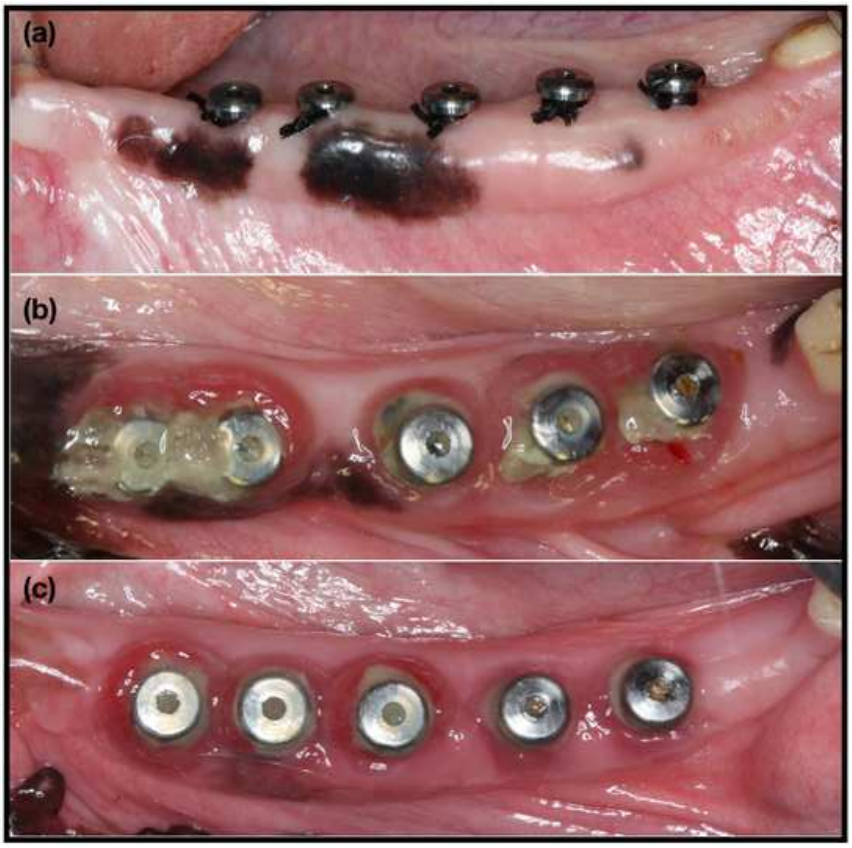


Figure 3

Click here to access/download;Figure;Fig 3.tif

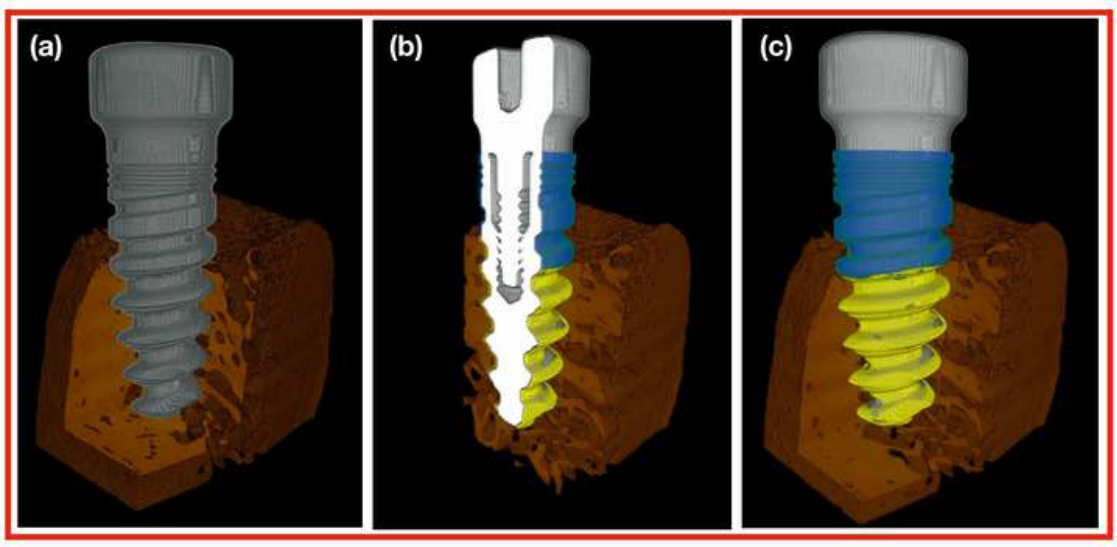


Figure 4

Click here to access/download;Figure;Fig 4.tif

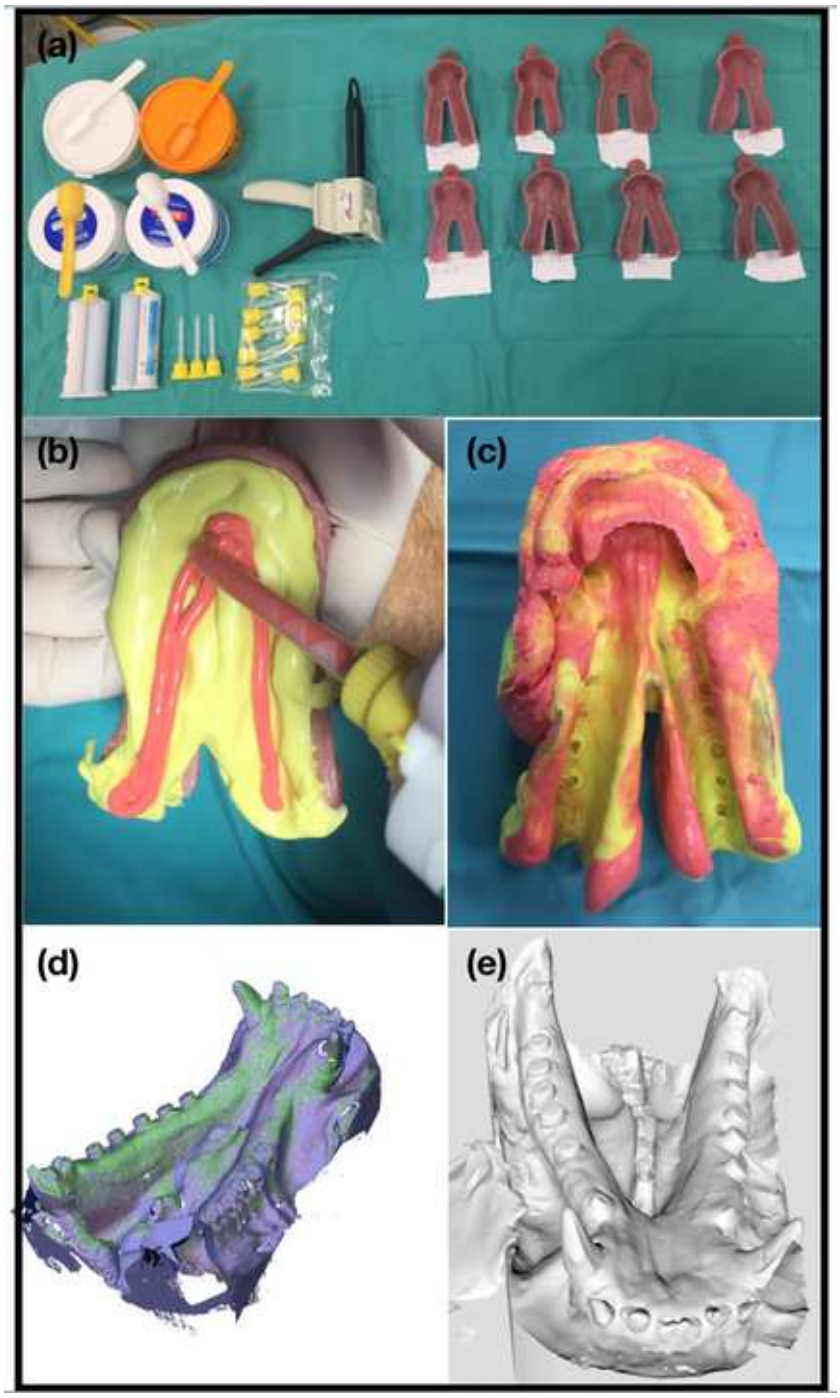
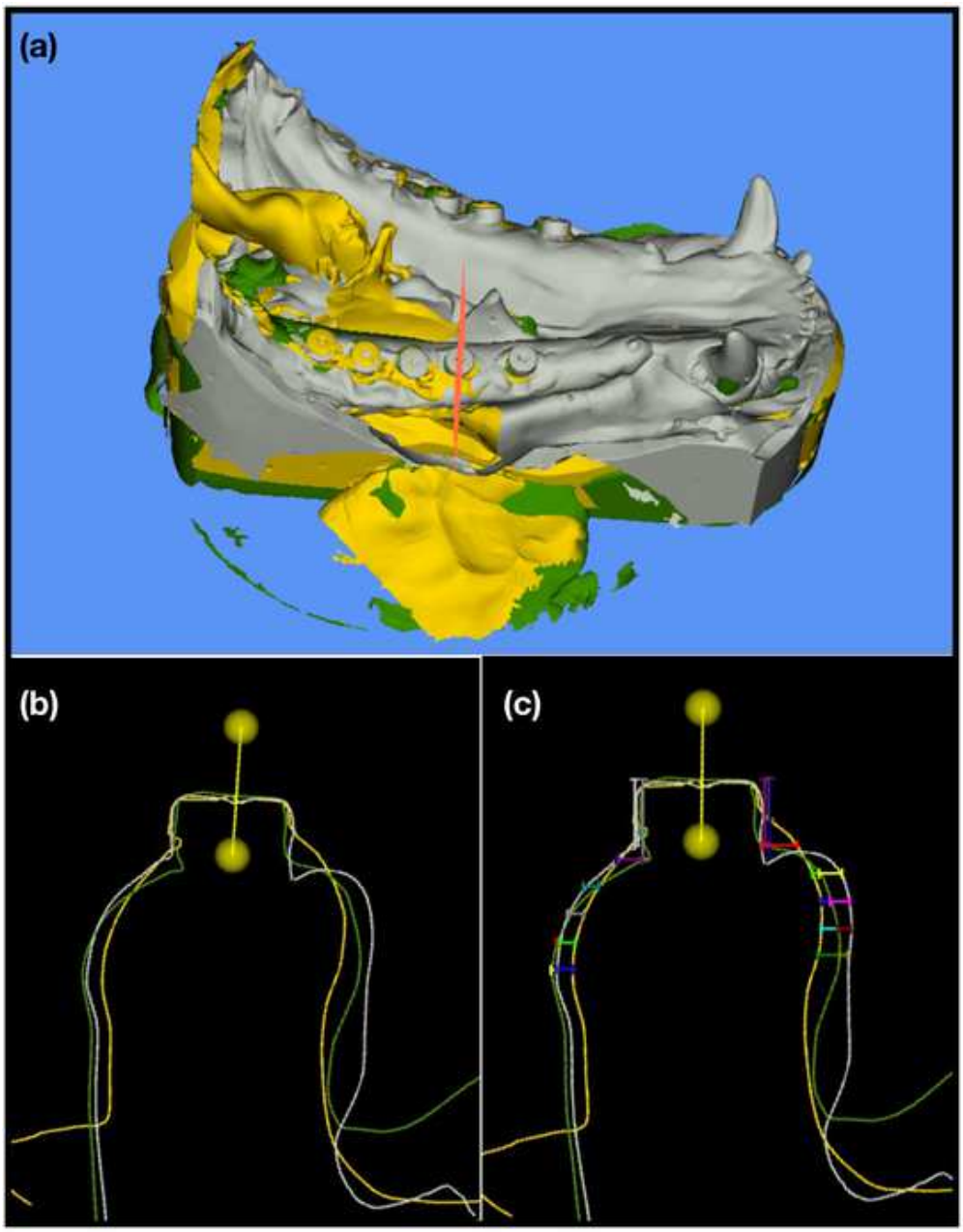


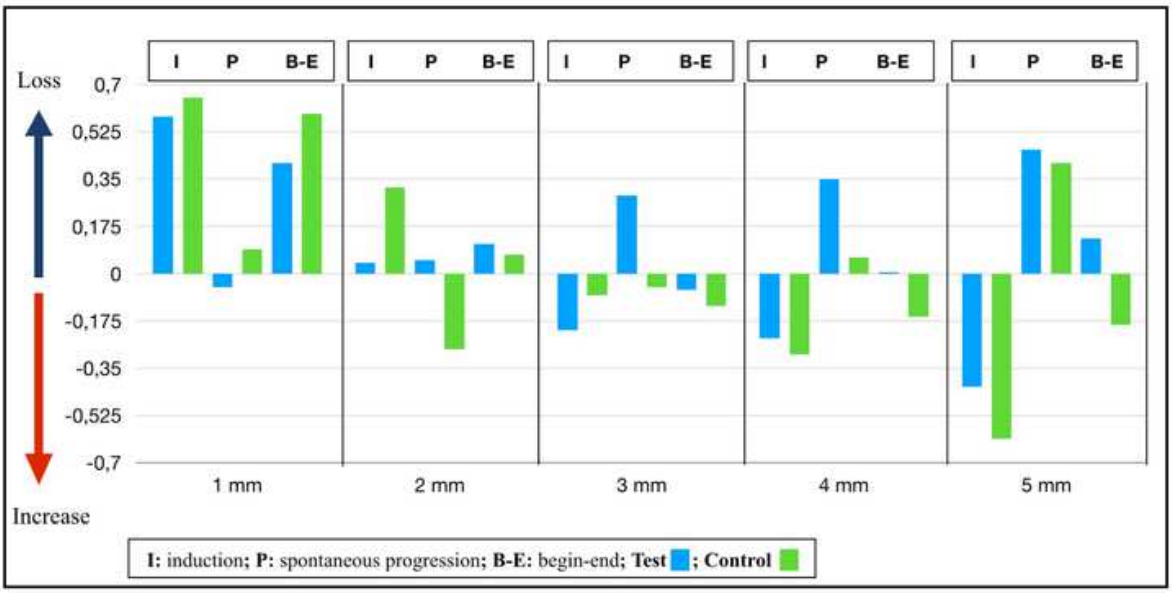
Figure 5

Click here to access/download;Figure;Fig 5.tif



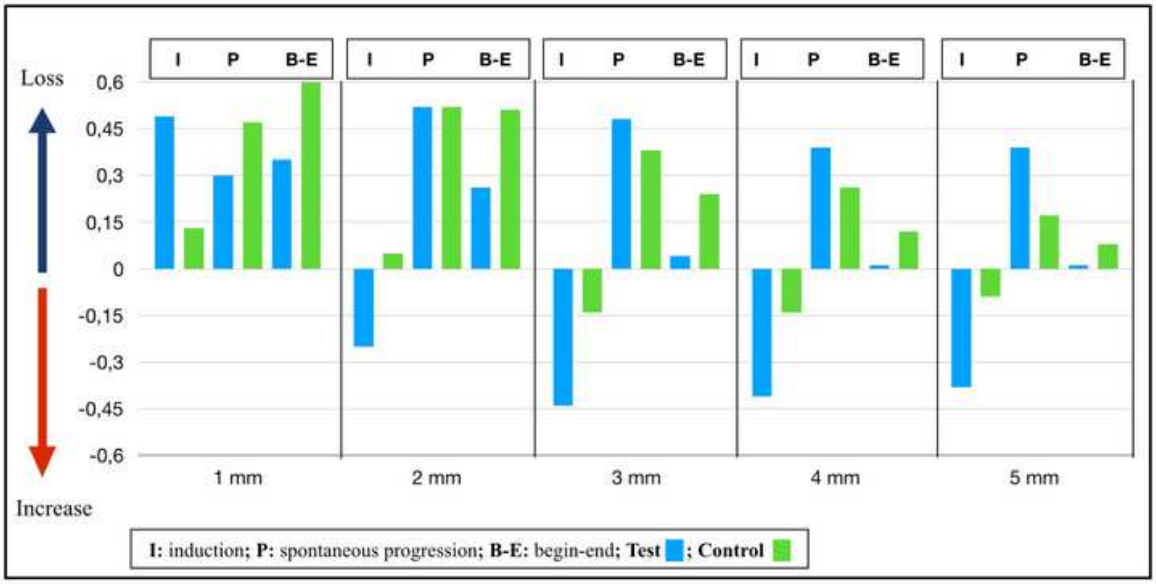
Graph 1

Click here to access/download;Figure;Graph 1 Horiz Buccal Soft Tissue.tif



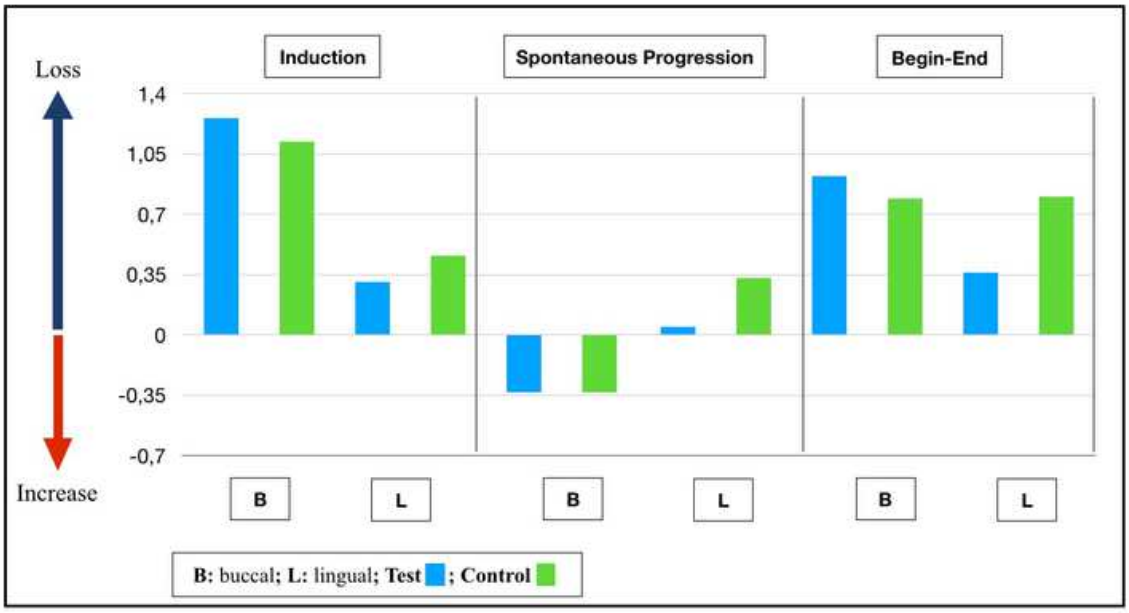
Graph 2

Click here to access/download;Figure;Graph 2 Horiz Lingual soft tissue.tif

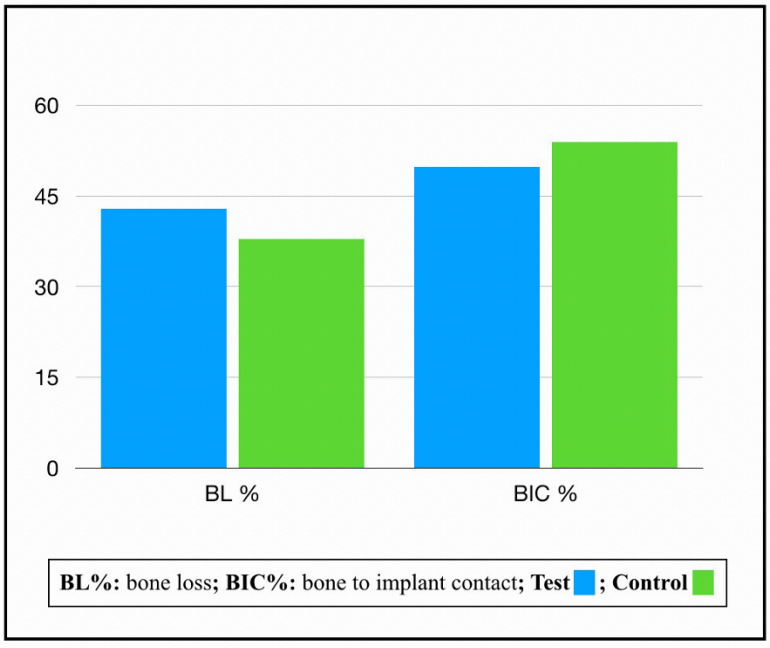


Graph 3

Click here to access/download;Figure;Graph 3 Vertical Soft Tissue.tif



Supplementary Graph 1



VIII. DISCUSIÓN

La interpretación de los resultados de los tres artículos de la presente tesis se hará en función del tipo de estudio en cuestión, ya que los primeros dos estudios son de ROG (Artículos 1 y 2), y el tercero de peri-implantitis, aunque en todos se usó el mismo modelo experimental preclínico in vivo. Además, mientras en el primer estudio de ROG (Artículo 1) se evaluó una nueva combinación de biomateriales (sustituto óseo sintético a base de BCP asociado a una membrana de colágeno entrecruzado), en el segundo estudio de ROG (Artículo 2) se evaluó una nueva membrana reabsorbible sintética a base de ácido poliláctico.

Con este fin, en primer lugar, se discutirán los resultados de los primeros dos estudios de ROG (Artículos 1 y 2) y posteriormente los resultados del estudio de peri-implantitis (Artículo 3).

Estudios de ROG

Las principales diferencias entre los estudios de ROG (Artículos 1 y 2), están en los materiales usados, además de los análisis realizados y los diferentes tiempos de seguimiento utilizados.

En el primer estudio (Artículo 1) se comparó como material test un sustituto óseo sintético particulado formado por HA (60%) + β-TCP (40%) asociado a una membrana de colágeno entrecruzado multicapa, comparado con un control positivo, consistente en el uso de un xenoinjerto (mineral óseo bovino desproteinizado, DBBM) asociado a una membrana de colágeno nativo y un control negativo donde no se colocó ningún material regenerativo.

En el segundo estudio (Artículo 2), mientras el control positivo fue igual que el anterior estudio (DBBM más una membrana de colágeno nativo), el grupo test consistía en el uso del mismo biomaterial de injerto óseo, pero asociado a una membrana sintética a base de ácido poliláctico, mientras en el control negativo se colocó solamente la membrana sintética a base de ácido poliláctico sin ningún sustituto óseo.

En cuanto a los análisis realizados, mientras que en el primer estudio (Artículo 1) se evaluaron solamente los cambios del contorno de los tejidos blandos, a las 8 y 16 semanas después de la

ROG, en el segundo estudio (Artículo 2) también se evaluaron los cambios volumétricos y del contorno de los tejidos duros y los tiempos de curación fueron 4 y 12 semanas.

Los resultados del primer estudio (Artículo 1) mostraron que no había diferencias significativas entre los grupos después de 8 semanas, sin embargo, a las 16 semanas, el grupo test y el control positivo mostraron un significativo mayor aumento del contorno de los tejidos blandos con respecto al control negativo.

En el segundo estudio (Artículo 2), a pesar que los materiales utilizados en la ROG difieren, se observó también que el aumento del contorno y del volumen de ambos tejidos blandos y duros fue superior cuando se utilizó una membrana asociada a un injerto óseo, en comparación con la membrana sola, después de ambos periodos de curación evaluados (4 y 12 semanas).

A partir de estos resultados, parece evidente el efecto beneficioso de combinar un injerto óseo y una membrana barrera en los procedimientos de ROG, así como confirman algunas revisiones sistemáticas previamente publicadas, donde se demuestra que el aumento óseo horizontal de crestas óseas atróficas con colocación simultánea de implantes es una técnica predecible y segura (Sanz Sanchez y cols 2015; Merli y cols. 2016; Wessing y cols. 2017; Thoma y cols. 2019). Además, se demostró que la tasa de supervivencia de implantes colocados en el hueso regenerado es similar a la correspondiente tasa de los implantes colocados en el hueso prístino (Donos y cols. 2008).

Si consideramos los sustitutos óseos utilizados en los procedimientos de ROG de ambos estudios de esta tesis (estudios 1 y 2), en los controles positivos se usó el mismo xenoinjerto bovino (DBBM). Este material ha sido ampliamente documentado en la literatura y a pesar de su lenta tasa de reabsorción es el más frecuentemente usado en regeneración ósea horizontal gracias a su capacidad osteoconductora. Sin embargo, otros sustitutos óseos como los injertos sintéticos, han mostrados resultados favorables y similares con respecto al DBBM gracias a sus propiedades bioactivas y a la posibilidad de regular su reabsorción (Sanz & Vignoletti 2015). De hecho, en la revisión de Bouler y cols., se confirmó que la degradación de los injertos óseos

sintéticos a base de BCP depende de la proporción de HA/β-TCP, además de defender el carácter osteoconductivo de estos materiales (Bouler y cols. 2017). En otra revisión los autores sugieren que estos materiales sintéticos a parte de ser osteoconductivos, poseen también capacidad osteoiductiva, ya que los fosfatos regulan la diferenciación y crecimiento de los osteoblastos y de los precursores de los osteoblastos a través de un aumento de la producción de IGF-1 y de BMPs (Jeong y cols. 2019). Sin embargo, la evidencia a respecto es limitada, al igual que el valor del "ratio" ideal de HA/β-TCP en los procedimientos regenerativos. Es por estas razones que diferentes estudios preclínicos y clínicos han comparados los BCP con diferentes "ratio" de HA/β-TCP entre ellos e incluso también con respecto al hueso autólogo, y al DBBM tras técnicas de regeneración ósea.

En varios estudios experimentales in vivo en un modelo de mini pig, los autores observaron después de procedimientos de ROG, mayor formación de nuevo hueso en los defectos tratados con hueso autólogo más una membrana no reabsorbible de e-PTFE, en comparación con los defectos en los cuales se usó la misma membrana de e-PTFE, pero asociada con el DBBM o con los BCP (Jensen y cols. 2006; Jensen y cols. 2009; Broggini y cols. 2015). Además, en los análisis histológicos e histomorfométricos se vio que el comportamiento de los BCP con porcentajes de HA/β-TCP (20/80) era similar al hueso autólogo, al igual que los BCP con porcentajes de HA/β-TCP (60/40) se comportaban de manera muy parecida al DBBM (Jensen y cols. 2006; Jensen y cols. 2009; Broggini y cols. 2015). Resultados similares se observaron en otra investigación preclínica en mini pig después de procedimientos de ROG comparando el DBBM con dos BCP con porcentajes diferentes de HA/β-TCP (90/10 y 60/40), y con todos los defectos cubiertos por una membrana de colágeno nativo (Dahlin y cols. 2015). En este estudio, los resultados volumétricos en términos de BV/TV obtenidos con el Micro-ct, pero también los datos histológicos e histomorfométricos, mostraron que a pesar de la superioridad en toda la investigación del BCP con el "ratio" de HA/β-TCP (90/10), mientras en las fases tempranas de la curación (3 semanas) el DBBM fue superior al BCP con el "ratio" de HA/β-TCP (60/40), a

las 8 semanas la cantidad de tejidos mineralizados fue similar (Dahlin y cols. 2015). Sin embargo, la observación más importante a nivel histológico en estos estudios fue que mientras las partículas de DBBM eran claramente visible en las distintas fases de la curación temprana y tardía, las partículas de los injertos a base de BCP presentaban diferentes grados de reabsorción y sustitución por nuevo hueso en función de su composición y obteniendo incluso mejores resultados con respecto al DBBM (Jensen y cols. 2006; Jensen y cols. 2009; Broggini y cols. 2015; Dahlin y cols. 2015).

Los resultados de los estudios experimentales in vivo mencionados hasta el momento (Jensen y cols. 2006; Jensen y cols. 2009; Broggini y cols. 2015; Dahlin y cols 2015), a pesar de confirmar la eficacia de los materiales a base de BCP en la ROG, no se pueden comparar directamente con los estudios de ROG de esta tesis por varias razones. Primero, mientras los defectos de los estudios de esta tesis eran crónicos (no contentivos), en todos los otros estudios los defectos eran de tipo agudo, lo que se traduce en defectos autocontentivos y con mayor vascularización, condiciones que facilitan la regeneración ósea. Además, a excepción del estudio de Dahlin y cols., en el cual se usaron membranas reabsorbibles de colágeno, en las otras investigaciones las membranas utilizadas eran no reabsorbibles, lo que se traduce con una mayor rigidez de las mismas; por último, también el diferente modelo experimental utilizado (perros vs mini pig), así como la metodología de análisis.

Sin embargo, otras investigaciones preclínicas han realizado procedimientos de ROG con estos materiales a base de BCP para tratar defectos crónicos de dehiscencia peri-implantarias al igual que nuestros estudios y los resultados fueron similares a los anteriores (Schwarz y cols. 2007; Lee y cols. 2016; Jung y cols. 2017).

En el estudio in vivo de Schwarz y cols., los autores compararon un xenoinjerto de origen bovina con respecto a un sustituto óseo a base de BCP con un porcentaje de HA/β-TCP (60/40), igual que el primer estudio de esta tesis, y, además, los defectos fueron recubiertos por el mismo tipo de membrana usada en los controles positivo de nuestros estudios, es decir una membrana

bicapa formada por colágeno tipo I y III (Schwarz y cols. 2007). Los datos histológicos mostraron que a pesar de no encontrar diferencias significativas entre estos materiales (BCP y DBBM) en las fases tempranas de curación (1 y 4 semanas), a las 9 semanas en los defectos en los cuales se usó el BCP había una mayor tasa de reabsorción de las partículas del biomaterial con una mayor sustitución por hueso nativo neo-formado, con respecto a los defectos tratados con el xenoinjerto donde quedaba un mayor número de partículas encapsuladas en las fibras del tejido conectivo (Schwarz y cols. 2007).

En el estudio de Lee y cols., los defectos de dehiscencia peri-implantarias se trataron con procedimientos de ROG combinando hueso autólogo más un BCP con un "ratio" de HA/β-TCP (70/30), con respecto al BCP solo. Además, los defectos fueron recubiertos por el mismo tipo de membrana de colágeno entrecruzado del grupo test del primer estudio de esta tesis (Lee y cols. 2016). A pesar de que los resultados histológicos fueron superiores en los grupos donde se usó la combinación de hueso autólogo con el BCP, sin embargo, en el análisis volumétrico con el Micro-ct las diferencias encontradas en términos de cantidad de tejido mineralizado y a la posición del primer contacto hueso-implante no fueron significativas entre los grupos. Un aspecto importante a considerar, es que en este estudio falta un grupo de estudio con la membrana de colágeno entrecruzado sola, el cual podría ayudar a entender mejor el comportamiento de esta membrana barrera (Lee y cols. 2016).

En el ensayo clínico aleatorizado (ECA) de Van Assche y cols., los defectos de dehiscencia en implantes se trataron con procedimientos de ROG comparando el DBBM con el β-TCP (Van Assche y cols. 2013). Los resultados después de 12 meses fueron similares entre ambos materiales, no sólo en cuanto a la reducción de la dehiscencia implantaria sino también en cuanto a la pérdida de hueso marginal alrededor de los mismos implantes y sin diferencias significativas. A pesar de la importancia de los resultados clínicos, esta investigación carece de evidencia histológica que permita analizar el comportamiento a nivel celular del biomaterial y su integración (Van Assche y cols. 2013).

A partir de los resultados comparativos de los estudios mencionados hasta el momento, se evidencia que la principal diferencia entre β-TCP y el DBBM es la tasa de reabsorción, además de la composición y calidad del nuevo hueso formado. De hecho, un estudio in vivo en un modelo en perros comparó las tasas de reabsorción del DBBM y del β-TCP, y observó que después de 3 meses en los grupos tratados con β-TCP las partículas de biomaterial se habían casi por completo reabsorbida hasta conseguir una completa neoformación ósea después de 24 meses, mientras las partículas de DBBM permanecieron sin reabsorber durante 24 meses (Artzi y cols. 2004). Estos resultados concuerdan con un estudio clínico que encontró a los 11 años después de procedimientos de elevación del seno maxilar, partículas de DBBM integradas con el hueso regenerado (Mordenfeld y cols. 2010).

Sin embargo, a pesar de la diferente tasa de degradación, los resultados del primer estudio de la presente tesis (Artículo 1), confirman que ambos sustitutos óseos (DBBM y BCP) son eficaces en la ROG, en cuanto se observó un aumento del contorno de la cresta después de ambos tiempos evaluados (8 y 16 semanas). Además, mientras a las 8 semanas las diferencias entre los grupos no fueron significativas, después de 16 semanas el grupo test fue significativamente superior en comparación con el control negativo en todas las alturas evaluadas (1, 3, 5 mm), al igual que el control positivo con respecto al control negativo, pero solo a 3 mm desde la parte coronal de la cresta.

Estos resultados han sido confirmados por los datos histológicos publicados en un artículo independiente donde se observó que en ambos tiempos de seguimientos (8 y 16 semanas) el test y el control positivos presentaban una mayor cantidad de nuevo hueso con respecto al control negativo, e incluso mostrando diferencias significativas a favor del test con respecto a los otros dos grupos después de 16 semanas (Jung y cols. 2017). En este estudio se observó además que mientras a las 8 semanas la membrana de colágeno nativo del control positivo se había casi en su totalidad degradada, completando su reabsorción a las 16 semanas, la membrana de colágeno entrecruzado del grupo test mantuvo su integridad estructural también a las 16 semanas (Jung

y cols. 2017), confirmando los resultados de una investigación previa donde tras realizar ROG en perros se vio que la membrana de colágeno nativo se había degradado después de 8 semanas mientras la membrana de colágeno entrecruzado siguió manteniendo su estructura después de 12 semanas (Cha y cols. 2017).

A partir de estos resultados, es posible, por lo tanto, que las diferencias encontradas entre test (BCP + membrana de colágeno entrecruzado) y el control positivo (DBBM + membrana de colágeno nativo) se hayan producido por los diferentes sustitutos óseos, pero también por las diferentes propiedades de las membranas, ya que la capacidad barrera de mantenimiento del espacio de las membranas puede influir en la cantidad de tejido regenerado (Polimeni y cols. 2005).

Si consideramos las membranas utilizadas en los estudios de ROG de esta tesis, mientras estas son diferentes entre los grupos test, sin embargo, la membrana del control positivo de ambos estudios (Artículos 1 y 2) es igual (colágeno nativo tipo I y III).

En cuanto a las membranas reabsorbibles de colágeno, éstas son las de elección en los procedimientos de ROG gracias a sus propiedades y a la amplia documentación científica y han sido utilizadas también en diferentes modelos animales (ratas y perros) con el objetivo de evaluar sus propiedades e incluso su tasa de degradación (Schwarz y cols. 2006; Schwarz y cols. 2008; Rothamel y cols. 2005; Owen & Yunkna 2001; Zhao y cols. 2000). En los resultados histológicos en un modelo in vivo en rata se observó que la degradación de estas membranas empieza después de solo 4 días tras su implantación, después de 10 días la membrana pierde su estructura y se fragmentan y terminan su degradación después de 21 días (Zhao y cols. 2000). Resultados similares han sido encontrados en este mismo modelo tras comparar membranas de colágeno nativo con respecto a membranas de colágeno entrecruzado (Rothamel y cols. 2005). En este estudio se observó en los análisis histológicos e histométricos que después de 2 semanas las membranas de colágeno nativo estaban por completo vascularizadas y que después de 4 semanas su grosor se había reducido significativamente, a diferencia de las membranas de

colágeno entrecruzado que seguían manteniendo su estructura después de 16 y 24 semanas (Rothamel y cols. 2005).

En un modelo en perros se observó que la degradación de las membranas de colágeno es ligera-moderada después de 1 - 2 meses, mientras a los 3 meses su degradación es más severa, hasta terminar después de 4 meses (Owen & Yukna 2001). Resultados similares se observaron en otro estudio en perros después de realizar procedimientos de ROG en defectos de dehiscencias peri-implantarias comparando membranas reabsorbibles de colágeno nativo con membranas de colágeno entrecruzado y con membranas no reabsorbibles (Schwarz y cols. 2008). Los resultados histológicos mostraron que después de solo 1 semana había una integración casi completa de las membranas de colágeno nativo con los tejidos circundantes y una completa degradación de las mismas después de 12 semanas. Sin embargo, el hallazgo histológico más importante de este estudio fue que las membranas de colágeno no sólo tenían la capacidad de promover migración celular y formación ósea, sino también favorecer la angiogénesis después de solo 1 semana tras su implantación, observación que fue además demostrada previamente por el mismo autor en otro modelo animal (rata) (Schwarz y cols. 2006; Schwarz y cols. 2008).

A pesar de estas ventajas, la rápida degradación de las membranas de colágeno nativo, en muchas ocasiones pueden resultar en un colapso de la misma en los defectos, lo que se traduce con una menor regeneración ósea. Sin embargo, las membranas de colágeno entrecruzado han demostrados mantener su estructura durante más tiempos, y aunque su capacidad angiogénica es menor, un ensayo clínico en humano ha demostrado su eficacia en los procedimientos de ROG, incluso observando resultados similares cuando se compararon con membranas no reabsorbibles (Friedmann y cols. 2002). De hecho, en este estudio, a pesar que los resultados histomorfométricos a los 7 meses mostraron una ligera superioridad de la membrana de colágeno entrecruzado, en términos de cantidad de tejido mineralizado, a nivel histológicos ambas membranas se comportaron de manera similar y sin diferencias significativas (Friedmann y cols. 2002). En otro ensayo clínico en humano, tras comparar membranas de

colágeno nativo con respecto a membranas de colágeno entrecruzado después de procedimientos de ROG en dehiscencias en implantes, se observó que a pesar de encontrar más dehiscencias de los tejidos blandos cuando se utilizaron membranas de colágeno entrecruzado, ambas membranas una vez expuestas promovían una cicatrización por segunda intención con la epitelización completa de la zona intervenida sin interferir en la regeneración ósea. Además, mientras las membranas de colágeno nativo se degradaron más rápidamente, las membranas de colágeno entrecruzado mantuvieron sus estructuras durante más tiempo, lo que se reflejó con una mayor cantidad de tejido mineralizado (Friedmann y cols. 2011).

Sin embargo, debido a los procesos de fagocitosis y degradación enzimática a los cuales son sometidas las membranas de colágeno entrecruzado, en muchas ocasiones, la aparición de reacciones inflamatorias e incluso infecciones secundarias en la zona intervenida son frecuentes (Bornstein y cols. 2007; Becker y cols. 2009), aunque en nuestro estudio (Artículo 1) no se observó ninguna reacción inflamatoria, ni exposición de la membrana o eventos de curación adversos.

Otra alternativa a las membranas reabsorbibles de colágeno para tratar los defectos óseos y con tiempos de degradación superiores a las membranas de colágeno nativo están representada por las membranas sintéticas. De hecho, en el segundo estudio de esta tesis (Artículo 2), los resultados fueron similares entre el test (membrana sintética a base de ácido poliláctico + DBBM) y el control positivo (membrana de colágeno nativo + DBBM), e incluso superiores a favor del grupo test, no sólo en términos de cambios volumétricos y del contorno de los tejidos duros, sino también en cuanto al aumento del contorno de los tejidos blandos. Además, en ambos tiempos de seguimientos (4 y 12 semanas), se observó que en los grupos donde se colocó solo la membrana test (grupo control negativo), hubo un aumento significativo de ambos tejidos blandos y duros, aunque con una extensión menor. Estos resultados podrían explicarse por la mayor rigidez de la membrana sintética, en comparación con la membrana de colágeno nativo,

lo que permite mantener el espacio para la regeneración más tiempo, además de la rápida tasa de degradación de las membranas de colágeno.

Resultados similares se observaron en un estudio in vivo en un modelo en perros después de realizar procedimientos de ROG en defectos de dehiscencia peri-implantarias comparando una membrana sintética a base de acido poliláctico con respecto a una membrana de colágeno (Won y cols. 2016). En este estudio, los resultados del análisis volumétrico, mediante Micro-ct mostraron mayores ganancias de nuevo hueso en los grupos donde se usó la membrana sintética, especialmente en el periodo de curación temprana (Won y cols. 2016). Además, cuando se usó la membrana de colágeno, fue frecuente encontrar partículas de biomateriales cerca del área de los defectos regenerados, condición que no se observó en los grupos con la membrana sintética, lo que confirma la mejor capacidad de las membranas sintéticas para mantener el espacio para la regeneración (Won y cols. 2016). La eficacia de las membranas sintéticas en los procedimientos de ROG ha sido evaluada también en otro estudio en perros donde se observó, en el análisis volumétrico, mayor ganancia de volumen y una mayor densidad ósea en los defectos en los cuales se utilizó la membrana sintética combinada con el factor de crecimiento fibroblástico (bFGF), en comparación con las mismas membranas sin el factor de crecimiento (Matsumoto y cols. 2012). Además, un hallazgo frecuente que se observó en este estudio, pero también en un ensayo clínico en humano, fue la alta prevalencia de inflamación de los tejidos blandos durante las fases finales de la degradación de la membrana sintética y su posterior exposición. Sin embargo, una vez expuestas, estas membranas, al igual que las membranas de colágeno, promovían una cicatrización por segunda intención y se cubrían gradualmente por tejidos mucosos sin alterar los resultados de la regeneración (Matsumoto y cols. 2012; Schneider y cols. 2014).

En el segundo estudio de esta tesis (Artículo 2), el mayor aumento del contorno de los tejidos blandos en el grupo test, en comparación con el control positivo, podría explicarse entonces por la mayor inflamación que genera la membrana sintética al degradarse. Sin embargo, en primer

lugar, no se observó ninguna exposición de la membrana, pero sobre todo los resultados del Micro-ct confirmaron la mayor cantidad de tejido mineralizado en el grupo test, en comparación con los otros grupos, lo que sugiere que las diferencias encontradas se deben a los diferentes tiempos de degradación de las membranas. De hecho, varios estudios preclínicos en monos han evaluados la tasa de reabsorción de la membrana sintética utilizada en nuestro estudio (Artículo 2), y observaron que su degradación empieza desde la capa interna hacia el exterior, lo que permite mantener su estructura después de 6 semanas, y completando su degradación después de 6-12 meses, a través de hidrólisis *(Figura 3)*, lo que deja en los tejidos de alrededor un ambiente ácido, que podría afectar la formación de nuevos tejidos (Gottlow y cols. 1994; Lundgren y cols. 1995). Sin embargo, en estos estudios, al igual que en el nuestro, no se observaron complicaciones ni efectos adversos, aunque los procedimientos fueron diferentes.

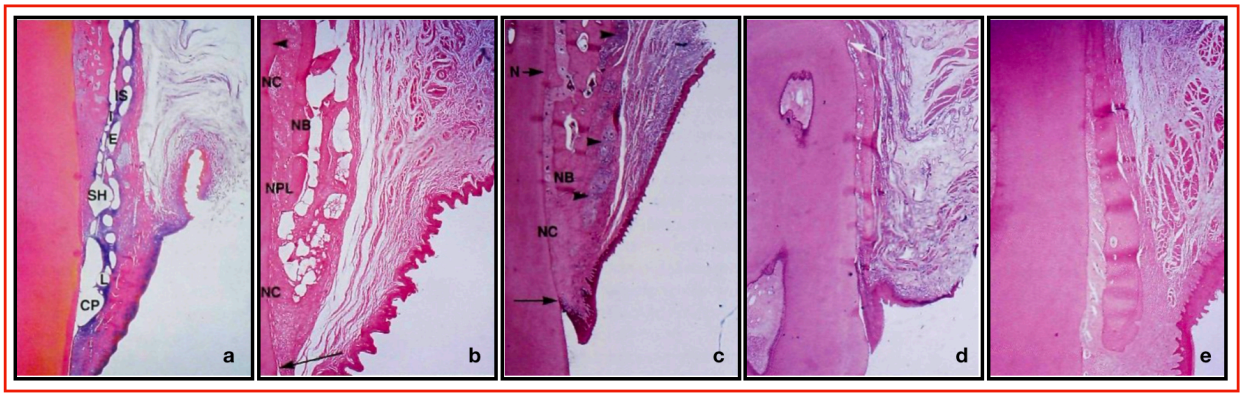


Figura 3. Representación gráfica de las distintas fases de degradación de la membrana sintética en los cortes histológicos. (a) A las 6 semanas la membrana está integrada por completo con los tejidos circundantes y mantiene su estructura (E= capa externa, I= capa interna, IS= espacio intermedio). (b) A los 3 meses la membrana empieza su degradación, pero al mismo tiempo se observa la formación de nuevo cemento (NC), ligamento periodontal (NPL) y hueso (NB). (c) A los 6 meses no es posible observar la estructura de la membrana, pero en cambio se observan macrofagos y células multinucleadas (flechas). (d) A los 12 meses se puede

observar tejido nuevo maduro y la ausencia de la membrana sintética. (e) Mayor maduración de los tejidos sin restos visibles de la membrana

Dentro de los resultados del segundo estudio de esta tesis (Artículo 2), uno de los más llamativos fue que también el control negativo mostró un aumento significativo de ambos tejidos blandos y duros, lo que podría sugerir que esta membrana sintética es capaz de mantener el espacio para la regeneración también sin el uso de ningún sustituto óseo debajo. Resultados similares se observaron en otros estudios preclínicos en un modelo en rata, que demostraron con el uso del Micro-ct, un aumento de nuevo hueso después de la ROG con la sola membrana y sin usar ningún biomaterial, sin embargo, la comparación directa con nuestro estudio no es posible, ya que los materiales son diferentes y en el nuestro estudio no se reportan datos histológicos (Beak y cols. 2016; Song y cols. 2014). Además, a pesar de la ganancia de ambos tejidos blandos y duros observada en el control negativo, en nuestro estudio, en los grupos donde se utilizó la combinación de un sustituto óseo y una membrana barrera (grupo test y control positivo), los resultados fueron superiores. Estos resultados concuerdan con cuanto observado en un ensayo clínico después de realizar procedimientos de ROG en defectos de dehiscencias y fenestración peri-implantarias con el mismo tipo de membrana sintética del grupo test de nuestro estudio, combinada o no con un injerto óseo autólogo (Lundgren y cols. 1994). A pesar que los resultados de esta serie de casos favorecieron los defectos donde se usó la combinación del injerto óseo con la membrana barrera, también en los defectos donde se usó la membrana sintética sola se observó un relleno óseo completo del defecto, lo que sugiere que esta membrana sintética, a pesar de tener excelentes propiedades de mantenedor de espacio, aún necesita combinarse con un biomaterial, especialmente en los defectos óseos no contentivos (Lundgren y cols. 1994).

El efecto beneficioso de combinar un injerto óseo con una membrana barrera para tratar defectos óseos no contentivos ha sido confirmado también en otro estudio in vivo después de

realizar procedimientos de ROG, pero sin la colocación de implantes (Sanz Martin y cols. 2017). Los resultados volumétricos y del contorno de los tejidos blandos medidos en modelos digitalizado, mostraron una superioridad cuando se usó un injerto óseo combinado o no con una membrana barrera, en comparación con la membrana sola, confirmando la importancia de usar un injerto óseo y una membrana barrera para los procedimientos de regeneración ósea (Sanz Martin y cols. 2017).

Los resultados del segundo estudio de esta tesis (Artículo 2), además, mostraron un resultado inesperado, especialmente en el análisis volumétrico y del contorno de los tejidos duros, con mayores ganancias de tejidos mineralizados después de 4 semanas de curación, en comparación con el largo período de seguimiento (12 semanas), independientemente de los grupos. Este hecho podría relacionarse con el diferente estado de degradación de las membranas, las cuales mientras después de 4 semanas ambas conservaban sus estructuras físicas completas o parciales, independientemente del tipo, por lo que la regeneración se mantuvo estable, sin embargo, después de 12 semanas, se completó la degradación de la membrana de colágeno y fue en etapas avanzadas para la membrana sintética, influyendo en la cantidad de nuevo hueso. Otra posible explicación en cuanto a la reducción del volumen de los tejidos duros entre el período de curación temprana (4 semanas) y tardía (12 semanas) podría relacionarse con el sobrecontorneado de los defectos con el xenoinjerto durante los procedimientos quirúrgicos regenerativos. Esto debería reflejarse entonces solamente en los grupos donde se usó el biomaterial, sin embargo, este hallazgo se observó en todos los grupos, lo que sugiere entonces que este hecho podría relacionarse con la contracción de los tejidos durante la cicatrización.

Estos resultados concuerdan con los datos reportados en un estudio preclínico en perros donde después de la ROG en defectos de dehiscencias peri-implantarias, se observó un mayor aumento del contorno vestibular de los tejidos duros en los tiempos de curación temprana vs tardía (Thoma y cols. 2017). Con estos resultados los autores sugieren que la formación de hueso sufre cambios estructurales después de los largos períodos de curación, pero también se observó una

menor formación ósea en el aspecto coronal de los defectos a causa del colapso de la membrana y al consecuente desplazamiento del biomaterial en una posición más apical (Thoma y cols. 2017). Éstas importantes consideraciones, se reflejan también en nuestro estudio (Artículo 2), ya que en ambos tiempos de cicatrización (4 y 12 semanas), en la mayoría de los defectos no se observó una completa regeneración a nivel de las dehiscencias peri-implantarias, independientemente de los grupos, y se observó que el primer contacto hueso-implante estaba en una posición más apical con respecto al hombro del implante (H= 0 mm), además de observar una mayor anchura de tejido mineralizado a nivel medio (H= 2 mm) y apical (H= 4 mm).

Estos resultados concuerdan también con los resultados de los tejidos blandos de ambos estudios de ROG de esta tesis (Artículos 1 y 2), ya que el aumento del contorno de los tejidos blandos tampoco fue uniforme a lo largo de la cresta alveolar vestibular, resultando menor a nivel coronal. De hecho, en ambos estudios de ROG (Artículos 1 y 2), todos los defectos se estratificaron en una parte coronal (1 mm por debajo de la cresta), intermedia (3 mm) y apical (5 mm) con el objetivo de evaluar a qué altura de la cresta se produjo el mayor aumento del contorno.

En el primer estudio (Artículo 1) después de ambos tiempos de cicatrización (8 semanas y 16 semanas), los resultados fueron superiores en el nivel intermedio, seguidos por el nivel apical y por último el nivel coronal. De manera similar, en el segundo estudio (Artículo 2), mientras después de 4 semanas se observó un mayor aumento a nivel apical, seguidos por el nivel intermedio y por último el nivel coronal, después de 12 semanas el mayor aumento se observó en el nivel intermedio, seguido por el nivel apical, pero nuevamente el menor cambio se observó a nivel coronal. Estos resultados podrían explicarse por la presión recibida en el aspecto coronal de la cresta durante la masticación de los perros, con un eventual parcial colapso de la membrana después de su aplicación, ya que en ambos estudios las membranas fueron fijadas con pin de titanio, apicalmente. Sin embargo, al igual que los datos histológicos del primer estudio de esta tesis mostraron un desplazamiento apical de los sustitutos óseos después de 8

semanas en los grupos donde se combinó el DBBM con la membrana de colágeno nativo (Jung y cols. 2017), también en el segundo estudio (Artículo 2) se observó en muchas secciones del Micro-ct el desplazamiento de algunas partículas de biomaterial apicalmente, justificando de esta manera los resultados del análisis de los tejidos blandos de ambos estudios (Artículos 1 y 2).

El desplazamiento de algunas partículas de biomaterial también se observó en varios estudios preclínicos después de procedimientos de ROG, tanto cuando se usaron pin de titanio para fijar las membranas, como también cuando no se usaron estos (Schwarz y cols. 2007; Sanz y cols. 2017; Mir-Mari 2017). De hecho, los autores explicaron que solo con el cierre de los colgajos después de los procedimientos regenerativos, algunas partículas se mueven apicalmente y es por esta razón que el aumento del volumen es menor en la porción coronal (Mir-Mari y cols. 2017).

En ambos estudios de ROG (Artículos 1 y 2), además, los defectos se estratificaron en mesial (A), central (B) y distal (C), así como se sugirió anteriormente en otra investigación in vivo (Sanz Martin y cols. 2017), con el objetivo de evaluar si la posición del defecto podría influir en los resultados de la regeneración. Los resultados de ambos estudios mostraron después de ambos periodos de cicatrización un mayor aumento del contorno de los tejidos blandos en los defectos mesiales en comparación con los defectos centrales y distales, aunque las diferencias no fueron significativas, confirmando que la posición no influye en los resultados regenerativos. Además, en el segundo estudio, cuando se estratificaron los defectos en función de los grupos de tratamiento (test, control positivo y negativo) y de su posición (A, B, C), los resultados mostraron una superioridad de ambos procedimientos de ROG (test y control positivo), en comparación con la membrana sola (control negativo), independientemente de la posición del defecto. Una vez más, estos resultados sugieren que no es la posición la que influye en los resultados de la regeneración, sino en los materiales utilizados.

Estudio de Peri-implantitis

La metodología utilizada en los estudios de ROG de esta tesis (Artículos 1 y 2) ha sido propuesta también en un modelo de peri-implantitis experimental después de comparar implantes con el mismo diseño y macroestructura pero con características de superficies diferentes, ya que los implantes test del tercer estudio (Artículo 3) se caracterizaban por una superficie bioactiva rica en fosfonatos.

Los resultados volumétricos obtenidos con el Micro-ct al final de la investigación mostraron una pérdida ósea pronunciada alrededor de todos los implantes, independientemente del grupo y sin diferencias significativas. En cuanto al análisis de los tejidos blandos, los resultados fueron diferentes en las distintas fases de la investigación, observando un aumento horizontal del contorno de los tejidos blandos al final de la inducción de la peri-implantitis, mientras se produjo una disminución horizontal del contorno durante la progresión espontánea, pero las diferencias entre los grupos no fueron tampoco significativas. A pesar de estos resultados, diferentes investigaciones preclínicas y clínicas han demostrado que la superficie bioactiva rica en multi-fosfonatos es capaz de promover el crecimiento óseo en la superficie del implante, con una mejor preservación del nivel óseo alrededor del mismo. De hecho, los resultados histológicos de un estudio in vivo mostraron que la superficie de los implantes con multi-fosfonatos presentaba una mayor osteointegración a las 2 semanas, en comparación con los implantes sin los fosfonatos. Además, a las 8 semanas había una mayor formación de nuevo hueso en los implantes con la superficie bioactiva, lo que se reflejó con un mayor contacto hueso-implante que se mantuvo incluso después de 52 semanas (Von Salis-Soglio y cols. 2014). Sin embargo, este tratamiento de superficie no tuvo un impacto positivo en los resultados de nuestra investigación, en cuanto no se encontraron diferencias significativas entre los implantes test y control, tanto en el análisis de los tejidos blandos, como en el análisis volumétrico de los tejidos duros, probablemente debido al diferente diseño de estudio (cresta ósea sana vs peri-

implantitis experimental), pero también por el diferente modelo usado (oveja vs perro), además de no presentar en nuestro estudio datos histológicos.

Los resultados del tercer estudio de esta tesis, de hecho, concuerdan con otro estudio de peri-implantitis experimental en perros, en el cual los autores tras comparar dos superficies de implantes con características diferentes encontraron diferencias significativas sólo a nivel histológico, pero no en el análisis volumétrico con el Micro-ct (Godoy-Gallardo y cols. 2016). El comportamiento de la superficie de los implantes test del tercer estudio de esta tesis, ha sido evaluado también en un estudio clínico en humano (Esposito y cols. 2013). En este estudio, los autores compararon implantes con la superficie de multi-fosfonatos con respecto a implantes con el mismo macro diseño, pero sin la superficie de multi-fosfonatos. Los resultados mostraron que no se perdió ningún implante, ni hubo complicaciones relacionadas con los mismos. Sin embargo, a los 3 y 12 meses después de sus cargas, a pesar de encontrar una menor pérdida de hueso marginal en los implantes con la superficie de multi-fosfonatos, las diferencias entre los dos grupos no fueron significativas (Esposito y cols. 2013). Estos resultados concuerdan con los resultados de nuestro estudio (Artículo 3), en cuanto no se encontraron diferencias entre los implantes test y control, tanto a nivel de los tejidos duros, como a nivel de los tejidos blandos, aunque no es posible comparar directamente estos datos ya que la metodología es diferente.

En cuanto a los resultados del análisis de los tejidos blandos, estos concuerdan además con cuanto observado a nivel clínico durante la investigación. De hecho, al final de la fase de inducción de la peri-implantitis a través de ligaduras, el aumento del contorno horizontal vestibular y lingual de los tejidos blandos en los STL, se reflejó clínicamente con un aumento del componente inflamatorio. Sin embargo, una vez retiradas las ligaduras y terminada la fase de progresión espontánea de la peri-implantitis, las mediciones horizontales del contorno de los tejidos blandos se redujeron, reflejándose clínicamente con la parcial resolución de la inflamación y una real pérdida de los tejidos blandos, independientemente del grupo. A nivel vertical, se observaron dehiscencias de los tejidos blandos, en toda la investigación,

independientemente del grupo, aunque éstas estaban más pronunciadas a nivel vestibular, en comparación con la parte lingual.

Una posible explicación podría relacionarse con la posición del nudo de las ligaduras, que en todos los implantes fue justo en vestibular. Por último, cuando se evaluaron los cambios del contorno horizontal de los tejidos blandos entre el inicio del estudio (antes de colocar las ligaduras) y el final, el comportamiento fue diferente entre el aspecto lingual y vestibular, independientemente de la superficie del implante, pero sin diferencias significativas.

A partir de estos resultados, parece evidente que mientras los resultados del Micro-ct confirman la abundante pérdida de hueso alrededor de todos los implantes, el comportamiento de los tejidos blandos es muy variable y no permite comparar directamente los resultados encontrados en los tejidos duros, sino que puede representar solamente una información adicional.

Sin embargo, numerosas investigaciones reportan resultados similares, en cuanto observaron en el mismo modelo de peri-implantitis experimental el componente inflamatorio a nivel de los tejidos blandos tras la colocación de ligaduras, pero también la pérdida de hueso alrededor de los implantes con peri-implantitis, aunque en estos estudios no se realizó análisis volumétricos y del contorno de los tejidos blandos y duros (Lindhe y cols. 1992; Berglundh y cols. 2007; Albouy y cols. 2008; Albouy y cols. 2009; Albouy y cols. 2012; Roehling y cols. 2019; Fickl y cols. 2015; Carcuac y cols. 2020; Battula y cols. 2015; Sanz-Esporrin y cols. 2019; Reinedahl y cols. 2018).

En un estudio clínico recientemente publicado, se evaluaron los cambios volumétricos de los tejidos blandos en los modelos digitalizados STL tras procedimientos regenerativos en peri-implantitis (Galarraga-Vinueza y cols. 2020). Los resultados mostraron después de 1 y 6 meses cambios evidentes a nivel de los tejidos blandos, especialmente en la parte coronal. A pesar de su carácter innovador, sin embargo, este estudio no es posible compararlo directamente con nuestras investigaciones, en cuanto no sólo los procedimientos son diferentes, sino también el modelo usado (estudio clínico vs preclínico), además de los análisis realizados (Galarraga-

Vinueza y cols. 2020). Es por esta razón que el tercer estudio de esta tesis (Artículo 3) se podría considerar como la primera investigación donde se realizó un análisis lineal del contorno de los tejidos blandos en un modelo de peri-implantitis.

En cuanto a la metodología utilizada en los tres estudios de esta tesis para evaluar los cambios de los tejidos blandos, a pesar de los diferentes métodos descritos en la literatura, sin embargo, el uso de escáneres ópticos se considera uno de los más confiable debido a la precisión de las mediciones y la objetividad de los resultados, y su eficacia ha sido confirmada en varios estudios preclínicos y también en ensayos clínicos, después de diferentes procedimientos quirúrgicos (Fickl y cols. 2008; Fickl y cols. 2009; Schneider y cols. 2011; Strebel y cols. 2009; Thoma y cols. 2010; Gonzalez-Martin y cols. 2014; Rebele y cols. 2014; Benic y cols. 2015; Sanz-Martin y cols. 2015; Schneider y cols. 2014; Jemt & Lekholm 2003; Jemt & Lekholm 2005; Henriksson y cols. 2004; Sanz-Martin y cols. 2016; Sanz-Martin y cols. 2017; Zeltner y cols. 2017; Sanz-Martin y cols. 2018; Basler y cols. 2018; Rojo y cols. 2018; Di Raimondo y cols. 2020).

Sin embargo, al igual que se mostró en nuestra primera investigación (estudio 1), estudiar solamente los tejidos blandos no explica el comportamiento de los tejidos duros, razón por la cual en el segundo y tercer estudios hemos evaluados también los cambios volumétricos de éstos, mediante análisis con Micro-ct. De hecho, la eficacia del Micro-ct para evaluar los cambios volumétricos de los tejidos duros, ha sido demostrada en varios estudios preclínicos y clínicos después de procedimientos regenerativos (Baek y cols. 2016; Al Hazmi y cols. 2013; Antunes y cols. 2015; Dahlin y cols. 2015; Won y cols. 2016; Matsumoto y cols. 2012; Hoornaert y cols. 2016; Li y cols. 2018; Becker y cols. 2017; Khobragade y cols. 2015; Beck-Broichsitter y cols. 2015; De Barros y cols. 2017; Lee y cols. 2015; Thoma y cols. 2017; Di Raimondo y cols. 2020), pero también en peri-implantitis (Varon-Shahar y cols. 2019; Maglione y cols. 2018; Godoy-Gallardo y cols. 2016; Qian y cols. 2019; Hiyari y cols. 2018).

Una importante observación obtenida con el Micro-ct en un estudio clínico después de la peri-implantitis inducida por ligaduras, fue la evidente alteración de la morfología del hueso periimplantario, cuando se evaluaron las características de las trabéculas óseas con respecto a la interfaz hueso-implante (Maglione y cols. 2018). Resultados similares se observaron en un estudio de peri-implantitis experimental en perros en el cual se propuso el uso del CBCT para evaluar las características de los defectos óseos periimplantarios, además de observar similitudes entre los defectos de peri-implantitis de los humanos y de los perros (Golubovic y cols. 2012).

Recientemente, se ha propuesto en la literatura combinar los archivos STL y las imágenes DICOM para estudiar de manera simultánea el comportamiento de ambos tejidos blandos y duros, aunque este método de análisis todavía tiene algunas limitaciones técnicas, con lo cual no es posible aplicarlo en todas las investigaciones (Sanz-Martin y cols. 2018).

A pesar de tratarse de estudios in vivo, los resultados de los tres estudios de esta tesis aportan varias informaciones útiles. En primer lugar, en cuanto a los procedimientos regenerativos, los resultados obtenidos sugieren que existen materiales alternativos en cuanto a sustitutos óseos y membranas, con respecto a los materiales que con más frecuencia se suelen usar en la ROG (xenoinjertos y membranas reabsorbibles de colágeno). De hecho, se vio que los materiales sintéticos a base de BCP con un "ratio" de HA/β-TCP (60/40) asociado a membranas de colágeno entrecruzado obtienen resultados similares, e incluso superiores, en comparación con el DBBM asociado a membranas de colágeno nativo (Artículo 1). También, se vio que las membranas sintéticas a base de ácido poliláctico, representan una válida alternativa a las membranas de colágeno nativo, en cuanto ambas membranas mostraron resultados similares cuando se combinaron con el DBBM (Artículo 2). En segundo lugar, se confirma la validez de estos métodos de análisis para evaluar los cambios de ambos tejidos blandos y duros también en la per-implantitis experimental. Sin embargo, la superficie de los implantes modificada con

los fosfonatos no aportó ventajas evidentes frente a la progresión de la peri-implantitis, ya que los resultados de ambas superficies fueron similares (Artículo 3).

A pesar de los resultados obtenidos en los distintos estudios, la metodología utilizada presenta algunas limitaciones y dificultades técnicas relacionadas con la obtención y procesamiento de los datos, pero también con los procesos de mediciones. En cuanto a la obtención y al procesamiento de los datos, éstos pueden tardar más tiempo respecto aquellos obtenidos directamente sobre el paciente en la clínica. Esto se debe a la necesidad de una preparación previa, es decir toma de impresiones, vaciado y escaneado para finalmente poder importar los archivos STL en aplicaciones específicas donde realizar las mediciones. También, para obtener mediciones correctas a nivel de los tejidos blandos, es necesario que las impresiones sean registradas lo mejor posible con todos los detalles anatómicos y que los modelos estudiados no tengan burbujas o irregularidades en el vaciado, ya que podrían falsear los resultados.

Además, ulteriores limitaciones se pueden encontrar cuando se consideran los estudios de manera individual.

En primer lugar, en el primer estudio de ROG (Artículo 1), la falta de datos volumétricos de los tejidos duros limita la interpretación de los resultados, aunque éstos han sido confirmados por los datos histológicos reportados en una publicación independiente (Jung y cols. 2017).

En el segundo estudio (Artículo 2), a pesar de evaluar ambos tejidos blandos y duros, sin embargo, debido a que el Micro-Ct trabaja con escalas de grises, en los análisis volumétricos de los tejidos duros no fue posible diferenciar entre el biomaterial y el hueso maduro, razón por la cual se analizaron ambos tejidos mineralizado en conjunto.

Además, la ausencia de datos histológicos limita la evaluación simultánea del comportamiento diferencial de los tejidos alrededor de los implantes en los distintos estudios, pero también el número reducido de las muestras (8 perros en cada estudio) podría limitar la significación de los resultados.

La última limitación de esta tesis, y que además se corresponde con la más importante, es que los tres estudios son preclínicos, por lo que no es posible traducir y comparar directamente los resultados obtenidos con situaciones clínicas reales, ya que las condiciones encontradas en los estudios experimentales en perros difieren de las condiciones observadas en los humanos.

A partir de estas consideraciones y de los resultados obtenidos y teniendo en cuenta del novedoso método propuesto para evaluar los cambios del contorno de los tejidos blandos en la peri-implantitis experimental, se ha podido confirmar la eficacia de la tecnología digital, en los distintos procedimientos quirúrgicos, también gracias a las congruencias de los datos obtenidos. Sin embargo, a pesar de las similitudes que comparten los perros y los humanos, hay que recordarse que la interpretación de los resultados es aproximativa, y habrá que confirmar cuanto observado en otros estudios preclínicos y clínicos, para comprender mejor el comportamiento de estos nuevos materiales (sustituto óseo y membranas), y también esta nueva superficie de implante.

Por último, sería interesante que las futuras investigaciones evalúan los tejidos blandos y duros de manera simultánea, con respecto a nuestras investigaciones donde se analizaron de manera independiente. La superposición de los archivos STL y de las imágenes DICOM y sus posteriores análisis simultáneas podrían ayudar a una mejor interpretación del comportamiento de ambos tejidos blandos y duros, con posibles más innovaciones futuras.

IX. CONCLUSIONES

A pesar de las limitaciones mencionadas anteriormente y de acuerdo a los objetivos descritos, es posible concluir que los métodos de análisis digital obtenidos a partir de la superposición de archivos STL y de imágenes DICOM del Micro-ct, son precisos y fiables para evaluar tanto los cambios del contorno de los tejidos blandos, como los cambios volumétricos de los tejidos duros, tras las diferentes intervenciones quirúrgicas (ROG y peri-implantitis experimental), además de confirmar el carácter no invasivo de estos métodos.

Si consideramos los objetivos específicos planteados al principio de esta tesis doctoral, las conclusiones son varias.

1. El uso de la tecnología digital en cuanto a la superposición de los archivos STL permitió demostrar que:
    a. Combinar un sustituto óseo y una membrana barrera permite obtener los mejores resultados en términos de aumento del contorno vestibular de los tejidos blandos tras ROG en defectos no contentivos (Artículos 1 y 2).
    b. No se encontraron diferencias significativas entre los grupos después de 8 semanas de curación, aunque el test y el control positivo fueron superiores. Sin embargo, después de 16 semanas, el grupo test (membrana de colágeno entrecruzado + BCP) y el control positivo (membrana de colágeno nativo + DBBM) obtuvieron un significativo mayor aumento del contorno de los tejidos blandos, en comparación con el control negativo (Artículo 1).
    c. Después de ambos periodos de seguimientos (4 y 12 semanas), a pesar de que el control negativo (membrana sintética sola) obtuvo un aumento significativo del contorno de los tejidos blandos, el grupo test (membrana sintética + DBBM) y el control positivo (membrana de colágeno nativo + DBBM) fueron comparables entre ellos y también superiores con respecto al control negativo, aunque las diferencias entre los grupos no fueron significativas (Articulo 2).

d. En ambos estudios de ROG, el mayor aumento del contorno de los tejidos blandos se observó a nivel apical y medio de la cresta, mientras el menor cambio fue a nivel coronal (Artículos 1 y 2)

e. El estudio de peri-implantitis experimental mostró que los cambios del contorno de los tejidos blandos fueron similares entre las dos superficies implantarias (test y control) en las diferentes fases del estudio, no solo a nivel horizontal sino también a nivel vertical, razones por cuales no es posible concluir que la superficie de los implantes test es superior con respecto a los implantes control. Sin embargo, esta investigación sigue siendo la primera que evaluó los cambios lineales del contorno de los tejidos blandos en un modelo de peri-implantitis experimental, con lo cual quizás podría abrir a una nueva manera de añadir más informaciones sobre la curación de los tejidos alrededor de los implantes con peri-implantitis (Artículo 3)

2. El análisis con el Micro-ct permitió evaluar los cambios volumétricos y del contorno de los tejidos duros en los dos modelos estudiados (ROG y peri-implantitis) mostrando que:

    a. Después de ambos periodos de seguimientos (4 y 12 semanas), a pesar de que la membrana sintética sola (control negativo) mostró un significativo aumento del volumen de los tejidos mineralizados, tanto los cambios volumétricos, como los cambios perfilométricos de los tejidos duros fueron superiores cuando se combinó un sustituto óseo y una membrana barrera (test y control positivo), aunque las diferencias entre los grupos no fueron significativas. Además, al igual que los tejidos blandos, también el aumento de los tejidos duros fue superior en la porción media y apical de la cresta ósea, en comparación con el nivel coronal que obtuvo el aumento menor (Artículo 2)

b. El estudio de peri-implantitis mostró que los cambios volumétricos de los tejidos duros de ambas superficies de implantes fueron similares, en términos de perdida ósea tridimensional y contacto hueso-implante (BIC%) y sin diferencias significativas, razones por las cuales no es posible concluir que los implantes test (superficie con multi-fosfonatos) son superiores en comparación con los implantes control, en términos de preservación del hueso peri-implantario (Artículo 3).

X. REFERENCIAS

1. Abrahamsson I, Berglundh T (2009) Effects of different implant surfaces and designs on marginal bone level alterations: a systematic review.Clin. Oral Impl. Res. 20 (Suppl. 4); 207–215.

2. Al-Askar, M., Javed, F., Al-Hezaimi, K., Al-Hamdan, K.S., Ramalingam, S., Aldahmash, A., Nooh, N., Al-Rasheed, A. (2016) Guided Bone Regeneration in Standardized Calvarial Defects in Rats Using Bio-Oss and β-Tricalcium Phosphate with Adjunct Platelet-Derived Growth Factor Therapy: A Real-Time In Vivo Microcomputed Tomographic, Biomechanical, and Histologic Analysis. Int J Periodontics Restorative Dent. 36 Suppl: s61-73

3. Albouy JP, Abrahamsson I, Berglundh T (2012) Spontaneous progression of experimental peri-implantitis at implants with different surface characteristics. An experimental study in dogs. J Clin Periodontol; 39: 182–187.

4. Albouy JP, Abrahamsson I, Persson LG, Berglundh T (2008) Spontaneous progression of peri-implantitis at different types of implants. An experimental study in dogs. I: clinical and radiographic observations. Clin Oral Implants Res.; 19 (10): 997- 1002.

5. Albouy JP, Abrahamsson I, Persson LG, Berglundh T (2009) Spontaneous progression of ligatured induced peri-implantitis at implants with different surface characteristics. An experimental study in dogs II: histological observations. Clin Oral Implants Res.; 20 (4): 366-71.

6. Al-Hazmi, B.A., Al-Hamdam, K.S., Al-Raheed, A., Babay, N., Wang, H.L., Al-Hezaimi, K. (2013) Efficacy of Using PDGF and Xenograft With or Without Collagen Membrane for Bone Regeneration Around Immediate Implants With Induced Dehiscence-Type Defects: A Microcomputed Tomographic Study in Dogs. J Periodontol. 84 (3): 371-8

7. Al-Hezaimi, K., Ramalingam, S., Al-Askar, M., ArRejaie, A.S., Nooh, N., Jawad, F., Aldahmash, A., Atteya, M., Wang, C.Y. (2016) Real-time-guided bone regeneration around standardized critical size calvarial defects using bone marrow-derived mesenchymal stem cells and collagen membrane with and without using tricalcium phosphate: an in vivo micro- computed tomographic and histologic experiment in rats. Int J Oral Sci. 30, 8 (1): 7-15

8. Amler MH (1969) The time sequence of tissue regeneration in human extraction wounds. Oral Surg Med Oral Pathol; 27 (3): 309-18

9. Antunes AA, Grossi-Oliveira GA, Martins-Neto EC, Almeida AL, Salata LA (2015) Treatment of circumferential defects with osseoconductive xenografts of different porosities: a histological, histometric, resonance frequency analysis, and micro-CT study in dogs. Clin Implant Dent Relat Res. 17 Suppl 1: e202-20

10. Araujo MG & Lindhe J (2005) Dimensional ridge alterations following tooth extraction. An experimental study in the dog. J Clin Periodontol 32: 212-218

11. Araujo MG & Lindhe J (2009) Ridge alterations following tooth extraction with and without flap elevation: an experimental study in the dog. Clin Oral Implants Res.; 20 (6):545-9.

12. Araujo, M.O., Berglundh, T., Lindhe, J. (1998) GTR treatment of degree III furcation defects with two different resorbable barriers. An experimental study in dogs. J Clin Periodontol. 25: 253-259.

13. Artzi, Z., Weinreb, M., Givol, N., Rohrer, M. D., Nemcovsky, C. E., Prasad, H. S., & Tal, H. (2004) Biomaterial Resorption Rate and Healing Site Morphology of Inorganic Bovine Bone and β-Tricalcium Phosphate in the Canine: A 24-month Longitudinal Histologic Study and Morphometric Analysis. International Journal of Oral & Maxillofacial Implants 19 (Suppl 3), 357-68

14. Asensio G, Vazquez-Lasa B, Rojo L (2019) Achievements in the Topographic Design of Commercial Titanium Dental Implants: Towards Anti-Peri-Implantitis Surfaces. J. Clin. Med.; 8 (11): 1982-2000

15. Baek YJ, Kim JH, Song JM, Zoon SY, Kim HS, Shin SH (2016) Chitin-fibroin-hydroxyapatite membrane for guided bone regeneration: micro-computed tomography evaluation in a rat model. Maxillofac Plast Reconstr Surg. 22; 38 (1): 14

16. Basler T, Naenni N, Schneider D, Hammerle CHF, Jung R, Thoma DS (2018) Randomized controlled clinical study assessing two membranes for guided bone regeneration of peri-implant bone defects: 3-year results. Clin Oral Implants Res. 29 (5): 499-507

17. Battula S, Lee JW, Wen HB, Papanicolaou S, Collins M, Romanos GE (2015) Evaluation of different implant designs in a ligature-induced peri-implantitis model: a canine study. Int J Oral Maxillofac Implants; 30: 534-545

18. Beck-Broichsitter BE, Garling A, Koehne T, Barvencik F, Smeets R, Mehl C, Becker ST (2015). 3D-tracking the regenerative potential of the mandible with micro-CTs. Oral and Maxillofacial Surgery, 19, 29–35.

19. Becker K, Klitzsch I, Stauber M, Schwarz F. (2017) Three- dimensional assessment of crestal bone levels at titanium implants with different abutment microstructures and insertion depths using micro-computed tomography. Clin. Oral Impl. Res. 28: 671–676.

20. Becker, J., Al-Nawas, B., Klein, M. O., Schliephake, H., Terheyden, H., & Schwarz, F. (2009). Use of a new cross-linked collagen membrane for the treatment of dehiscence-type defects at titanium implants: a prospective, randomized-controlled double-blinded clinical multicenter study. Clinical Oral Implants Research 20 (Suppl 7), 742-749.

21. Benic GI & Hämmerle CHF (2014) Horizontal bone augmentation by means of guided bone regeneration. Periodontology 2000; 66:13-40

22. Benic GI, Elmasry M, Hammerle CH (2015) Novel digital imaging techniques to assess the outcome in oral rehabilitation with dental implants: a narrative review. Clin Oral Implants Res.; 26 Suppl 11: 86-96.

23. Berglundh T, Lindhe J, Ericsson I, Marinello CP, Liljeberg B, Thomsen P (1991) The soft tissue barrier at implants and teeth. Clin Oral Impl Res; 2: 81-90

24. Berglundh T, Armitage G, et al. (2018) Peri- implant diseases and conditions: Consensus report of workgroup 4 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Clin Periodontol.;45(Suppl 20): S286–S291.

25. Berglundh T, Gislason O, Lekholm U, Sennerby L, Lindhe J (2004) Histopathological observations of human periimplantitis lesions. J Clin Periodontol; 31: 341–347.

26. Berglundh T, Gotfredsen K, Zitzmann NU, Lang NP, Lindhe J (2007) Spontaneous progression of ligature induced peri-implantitis at implants with different surface roughness: an experimental study in dogs. Clin Oral Implants Res.; 18(5): 655- 61.

27. Berglundh T, Zitzmann NU, Donati M (2011) Are peri-implantitis lesions different from periodontitis lesions? J Clin Periodontol; 38 (Suppl. 11): 188–202

28. Bernhardt R, Kuhlisch E, Schulz MC, Eckelt U, Stadlinger B (2012) Comparison of bone-implant contact and bone-implant volume between 2D-Histological sections and 3D-SRmicroCT slice. European Cells and Materials; 23: 237-248

29. Binsalah, M.A., Ramalingam, S., Alkindi, M., Nooh, N., Al-Hezaimi, K. (2019) Guided Bone Regeneration of Femoral Segmental Defects using Equine Bone Graft: An In-Vivo Micro- Computed Tomographic Study in Rats. J Invest Surg 32 (5): 456-466

30. Bornstein, M. M., Bosshardt, D., & Buser, D. (2007). Effect of two different bioabsorbable collagen membranes on guided bone regeneration: a comparative histomorphometric study in the dog mandible. Journal of Periodontology 78 (Suppl 10), 1943-1953.

31. Bouler JM, Pilet P, Gauthier O, Verron E (2017) Biphasic calcium phosphate ceramics for bone reconstruction: A review of biological response. Acta Biomaterialia; 53: 1-12

32. Boyan BD, Batzer R, Kieswetter K, Liu Y, Cochran DLK, et al. (1998). Titanium surfaces roughness alters responsiveness of MG63 osteoblast-like cells to 1 alfa,25-(OH)2 D3. J Biomed Mater Res.; 39: 77-85

33. Broggini N, Bosshardt DD, Jensen SS, Bornstein MM, Wang CC, Buser D (2015) J Biomed Mater Res B App Biomat; 103 (7): 1478-87

34. Brunski JB, Puleo DA, Nanci A (2000). Biomaterials and biomechanics of oral and maxillofacial implants: current status and future developments. Int J Oral Maxillofac Implants; 15: 15-46.

35. Caneva M, Salata LA, de Souza SS, Baffone G, Lang NP, Botticelli D (2010) Influence of implant positioning in extraction sockets on osseointegration: histomorphometric analyses in dogs. Clin. Oral Impl. Res. 21: 43–49

36. Carcuac O, Abrahamsson I, Derks J, Petzold M, Berglundh T (2020) Spontaneous progression of experimental peri-implantitis in augmented and pristine bone. A pre-clinical in vivo study. Clin Oral Implants Res.; 31(2):192-200

37. Cardaropoli G, Araujo M, Lindhe J (2003) Dynamics of bone tissue formation in tooth extraction sites. An experimental study in dogs. J Clin Periodontol; 30: 809–818

38. Cardaropoli G, Lekholm U, Wennstrom JL (2006) Tissue alterations at implant-supported single-tooth replacements: a 1-year prospective clinical study. Clin Oral Implants Res.;17 (2): 165-71.

39. Carpio L, Loza J, Lynch S, Genco R (2000) Guided bone regeneration around end osseous implants with inorganic bovine bone mineral. A randomised controlled trial comparing bioabsorbable versus non-reabsorbable barriers. J Periodontol; 71: 1743-1749

40. Carrasco-García A, Castellanos-Cosano L, Rodríguez-Pérez A, Corcuera-Flores JR, Torres-Lagares D, Machuca-Portillo G (2019) Influence of marginal bone loss on peri-implantitis: Systematic re-view of literature. J Clin Exp Dent.;11(11): e1045-71

41. Caton J, Armitage G, Berglundh T, et al. (2018) A new classification scheme for periodontal and peri- implant diseases and conditions – Introduction and key changes from the 1999 classification. J Clin Periodontol.;45(Suppl 20): S1–S8.

42. Cha JK, Joo M-J, Yoon S, Lee J-S, Choi S-H, Jung U-W (2017) Sequential healing of onlay bone grafts using combining biomaterials with cross-linked collagen in dogs. Clin. Oral Impl. Res.; 28: 76–85

43. Chappuis V, Engel O, Shahim K, Reyes M, Katsaros C, Buser D (2015) Soft tissue alterations in esthetic postextraction sites: A 3-Dimensional analysis. J Dent Res; 94 (9 Suppl): 187S-93S

44. Chappuis V, Araujo MG, Buser D (2017) Clinical relevance of dimensional bone and soft tissue alterations post-extraction in esthetic sites. Periodontology 2000; 73 (1): 73-83

45. Chen LC, Lundgren T, Hallstrom H, Cherel F (2008) Comparison of different methods of assessing alveolar ridge dimensions prior to dental implant placement. J Periodontol.;79 (3): 401-5.

46. Cooper LF, Masuda T, Whiston SW, Yliheikkila P, Felton DA (1999). Formation of mineralizing osteoblast cultures on machined titanium surface. Int J Maxilllofac Implants.; 14: 37-34.

47. Dahlin C, Linde A, Gottlow J & Nyman S (1988) Healing of bone defects by guided tissue regeneration. Plast Reconstr Surg 81: 672-676

48. Dahlin, C., Obrecht, M., Dard, M., Donos, N. (2015) Bone tissue modelling and remodelling following guided bone regeneration in combination with biphasic calcium phosphate materials presenting different microporosity. Clin. Oral Impl. Res. 26 (7): 814-22

49. de Barros RRM, Novaes AB Jr, de Carvalho JP, de Almeida ALG (2017) The effect of a flapless alveolar ridge preservation procedure with or without a xenograft on buccal bone crest remodeling compared by histomorphometric and microcomputed tomographic analysis. Clin. Oral Impl. Res. 28: 938–945

50. Derks J & Tomasi C. Peri-implant health and disease (2015) A systematic review of current epidemiology. J Clin Periodontol; 42 (Suppl. 16): S158–S171.

51. Di Raimondo R, Sanz-Esporrin J, Pla R, Sanz-Martin I, Luengo F, Vignoletti F, Nuñez J, Sanz M (2020) Alveolar crests contour changes after guided bone regeneration using different biomaterials: an experimental in vivo investigation. Clin Oral Investig; 24 (7): 2351-2361

52. Di Raimondo R, Sanz-Esporrin J, Sanz-Martin I, Pla R, Luengo F, Vignoletti F, Nuñez J, Sanz M (2020) Hard and soft tissue changes after guided bone regeneration using two different barrier membranes: an experimental in vivo investigation. Clin Oral Investig; doi: 10.1007/s00784-020-03537-5

53. Donos N, Mardas N, Chadha V (2008) Clinical outcomes of implants following lateral bone augmentation: systematic assessment of available options (barrier membranes, bone grafts, split osteotomy). J Clin Periodontol; 35 (Suppl. 8): 173–202

54. Draper HH (1994) Bone loss in animals. In: Draper HH ed. Advances in Nutritional Research; Vol. 9: 53–71

55. Elgali, I., Omar, O., Dahlin, C., Thomsen, P. (2017) Guided bone regeneration: materials and biological mechanisms revisited. Eur J Oral Sci. 125: 315–337.

56. Esposito M, Dojcinovic I, Germon L, Lévy N, Curno R, Buchini S, Péchy P, Aronsson BO (2013). Safety and efficacy of a biomimetic monolayer of permanently bound multi-phosphonic acid molecules on dental implants: 1 year post-loading results from a pilot quadruple-blinded randomised controlled trial. Eur. J. Oral Implantol.; 6: 227–236.

57. Farmer M & Darby I (2014). Ridge dimensional changes following single-tooth extraction in the aesthetic zone. Clin. Oral Impl. Res; 25: 272–277

58. Fickl S, Kebschull M, Calvo-Guirado JL, Hurzeler M, Zuhr O (2015) Experimental Peri-Implantitis around Different Types of Implants – A Clinical and Radiographic Study in Dogs. Clin Implant Dent Relat Res.;17 Suppl 2: e661-9

59. Fickl S, Schneider D, Zuhr O, Hinze M, Ender A, Jung RE (2009) Dimensional changes of the ridge contour after socket preservation and buccal overbuilding: an animal study. Journal of Clinical Periodontology 36 (Suppl 5):442-8.

60. Fickl S, Zuhr O, Wachtel H, Bolz W, Huerzeler M (2008) Tissue alterations after tooth extraction with and without surgical trauma: a volumetric study in the beagle dog. J Clin Periodontol.; 35 (4): 356-63.

61. Fickl S, Zuhr O, Wachtel H, Stappert CF, Stein JM, Hurzeler MB (2008) Dimensional changes of the alveolar ridge contour after different socket preservation techniques. J Clin Periodontol.; 35 (10): 906-13.

62. Finelle G, Papadimitriou DEV, Souza AB, Katebi N, Gallucci GO, Araujo MG (2015) Peri-implant soft tissue and marginal bone adaptation on implant with non-matching healing abutments: micro-CT analysis. Clin. Oral Impl. Res.; 26: e42–e46

63. Friedmann A, Strietzel FP, Maretzki B, Pitaru S, Bernimoulin JP (2002) Histological assessment of augmented jaw bone utilizing a new collagen barrier membrane compared to a standard barrier membrane to protect a granular bone substitute material Clin. Oral Impl. Res; 13: 587–594

64. Friedmann A, Gissel K, Soudan M, Kleber B-M, Pitaru S, Dietrich T (2011) Randomized controlled trial on lateral augmentation using two collagen membranes: morphometric results on mineralized tissue compound. J Clin Peridontol; 38: 677–685

65. Galarraga-Vinueza ME, Obreja K, Magini R, Sculean A, Sader R, Schwarz F (2020). Volumetric assessment of tissue changes following combined surgical therapy of peri-implantitis: A pilot study. J Clin Periodontol.;00:1–10. https://doi.org/10.1111/jcpe.13335

66. Gentile, P., Chiono, V., Tonda-Turo, C., Ferreira, A.M., Ciardelli, G. (2011) Polymeric membranes for guided bone regeneration. Biotechnol J. 6 (10): 1187-97

67. Godoy-Gallardo M, Manzanares-Cespedes MC, Sevilla P, Nart J, Manzanares N, Manera JM., Gil FJ, Body SK, Rodriguez D (2016) Evaluation of bone loss in antibacterial coated dental implants: An experimental study in dogs. Mater Sci Eng C Mater Biol Appl. 1; 69: 538-45.

68. Golubovic V, Mihatovic I, Becker J, Schwarz F (2012) Accuracy of cone-beam computed tomography to assess the configuration and extent of ligature-induced peri-implantitis defects. A pilot study. Oral Maxillofac; 16: 349-354

69. Gonzalez-Martin O, Veltri M, Moraguez O, Belser UC (2014) Quantitative three-dimensional methodology to assess volumetric and profilometric outcome of subepithelial connective tissue grafting at pontic sites: a prospective pilot study. Int J Periodontics Restorative Dent.; 34 (5): 673-9.

70. Gottlow J, Laurell L, Lundgren D, Mathisen T, Nyman S, Rylander H, Bogentoff C (1994) Periodontal tissue response to a new bioresorbable guided tissue regeneration device: a longitudinal study in monkeys. Int J Periodont Rest Dent; 14: 437-449

71. Grunder U, Gracis S, Capelli M (2005) Influence of 3-D bone-to-implant relationship on esthetics. Int J Periodontics Restorative Dent; 25: 113-119

72. Guarnieri, R., Stefanelli, L., De Angelis, F., Mencio, F., Pompa, G., & Di Carlo, S. (2017). Extraction socket preservation using porcine- derived collagen membrane alone or associated with porcine-derived bone. Clinical Results of Randomized Controlled Study. Journal of Oral & Maxillofacial Research. 30; 8 (3): e5.

73. Guarnieri, R., Testarelli, L., Stefanelli, L., De Angelis, F., Mencio, F., Pompa, G., & Di Carlo, S. (2017). Bone healing in extraction sockets covered with collagen membrane alone or associated with porcine- derived bone graft: A comparative histological and histomorphometric analysis. Journal of Oral & Maxillofacial Research. 31; (4): e4.

74. Hammerle CH, Jung RE, Yaman D, Lang NP (2008) Ridge augmentation by applying bioresorbable membranes and deproteinized bovine bone mineral: a report of twelve consecutive cases. Clin Oral Implants Res.; 19 (1): 19-25.

75. Hammerle CH & Jung RE (2003) Bone augmentation by means of barrier membranes. Periodontol 2000; 33: 36-53.

76. Hammerle CHF & Tarnow D (2018) The etiology of hard and soft tissue deficiencies at dental implants: A narrative review. J Clin Periodontol. 45 (Suppl 20): S267–S277

77. Haugen HJ, Lyngstadaas SP, Rossi F, Perale G (2019) Bone grafts: which is the ideal biomaterial? J Clin Periodontol. 46 (Suppl. 21): 92–102

78. Heitz-Mayfield LJA, Salvi GE (2018) Peri-implant mucositis. J Clin Periodontol.;45 (Suppl 20): S237–S245.

79. Henriksson K & Jemt T (2004) Measurements of soft tissue volume in association with single implant restoration: a 1 year comparative study after abutment connection surgery. Clin Implant Dent Relat Res 6(4): 181–189

80. Hiyari S, Wong RL, Yaghsezian A, et al. (2018) Ligature-induced peri-implantitis and periodontitis in mice. J Clin Periodontol.;45:89–99.

81. Hoornaert A, d'Arros C, Heymann MF, Layrolle P (2016) Biocompatibility, resorption and biofunctionality of a new synthetic biodegradable membrane for guided bone regeneration. Biomed Mater. 11 (4): 045012

82. Huang Y, Li Z, Van Dessel J, Salmon B, Huang B, Lambrichts I,Politis C, Jacobs R (2019) Effect of platelet-rich plasma on peri-implant trabecular bone volume and architecture: A preclinical micro-CT study in beagle dogs. Clin Oral Impl Res.; 30: 1190–1199

83. Iasella JM, Greenwell H, Miller RL, Hill M, Drisko C, Bohra AA, Scheetz JP (2003) Ridge Preservation with Freeze-Dried Bone Allograft and a Collagen Membrane Compared to Extraction Alone for Implant Site Development: A Clinical and Histologic Study in Humans. J Periodontol; 74: 990-999

84. Ivanosky S & Lee R (2017) Comparison of peri-implant and periodontal marginal soft tissues in health and disease. Periodontology 2000; 0: 1-15

85. Jansen JA, Brugge P, Van Der Waal E, Vredenberg A, Wolke J (2003). Osteocapacities of calcium phosphate ceramics. In: Ellingsen JE, Lyngstadaas SP (eds). Bioimplant Inter- face. Boca Raton, FL: CRC:305-22.

86. Jemt T & Lekholm U (2003) Measurements of buccal tissue volumes at single-implant restorations after local bone grafting in maxillas: a 3-year clinical prospective study case series. Clinical Implant Dentistry & Related Research 5 (Suppl 2): 63-70.

87. Jemt T & Lekholm U (2005) Single implants and buccal bone grafts in the anterior maxilla: measurements of buccal crystal contours in a 6 year prospective clinical study. Clinical Implant Dentistry & Related Research; 7 (3): 127-35

88. Jensen SS, Broggini N, Hj□rting-Hansen E, Schenk R, Buser D (2006) Bone healing and graft resorption of autograft, anorganic bovine bone and b-tricalcium phosphate. A histologic and histomorphometric study in the mandibles of minipigs. Clin. Oral Impl. Res.;17: 237–243

89. Jensen SS, Yeo A, Dard M, Hunziker E, Schenk R, Buser D (2007). Evaluation of a novel biphasic calcium phosphate in standardized bone defects. A histologic and histomorphometric study in the mandibles of minipigs. Clin. Oral Impl. Res.; 18: 752–760

90. Jensen SS, Bornstein MM, Dard M, Bosshardt DD, Buser D (2009) Comparative Study of Biphasic Calcium Phosphates With Different HA/TCP Ratios in Mandibular Bone Defects. A Long-Term Histomorphometric Study in Minipigs. J Biomed Material Research; 90B (1): 171-181

91. Jeong J, Kim JU, Shim JH, Hwang S, Hey CY (2019) Bioactive calcium phosphate materials and applications in bone regeneration. Biomaterials Research; 23 (4): 1-11

92. Jordana F, Susbielles L, Colat-Parros J (2018) Periimplantitis and implant body roughness: a systematic review of literature. Implant Dentistry; 27 (6): 672-681

93. Jung RE, Pjetursson BE, Glauser R, Zembic A, Zwahlen M, Lang NP (2008) A systematic review of the 5-year survival and complication rates of implant-supported single crowns. Clin. Oral Impl. Res. 19; 119–130

94. Jung RE, Zembic A, Pjetursson BE, Zwahlen M, Thoma DS (2012) Systematic review of the survival rate and the incidence of biological, technical and esthetic complications of single crowns on implants reported in longitudinal studies with a mean follow-up of 5 years. Clin. Oral Implants Res. 23(Suppl. 6), 2–21

95. Jung RE, Fenner N, Ha¨ mmerle CHF, Zitzmann NU (2013) Long-term outcome of implants placed with guided bone regeneration (GBR) using resorbable and non-resorbable membranes after 12–14 years. Clin. Oral Impl. Res.;24: 1065–1073

96. Jung UW, Cha JK, Vignoletti F, Nunez J, Sanz J, Sanz M (2017) Simultaneous lateral bone augmentation and implant placement using a particulated synthetic bone substitute around chronic peri-implant dehiscence defects in dogs. J Clin Periodontol 44: 1172-1180

97. Khobragade P, Jain A, Setlur Nagesh SV, Andreana S, Dziak R, Sunkara SK, Ionita CN (2015). Micro-Computed tomography (CT) based assessment of dental regenerative therapy in the canine mandible model. Proceedings of SPIE-the International Society for Optical Engineering, 17, 9417

98. Kieswetter K, Schwartz Z, Hummert TW, Cochran DL, Simpson J (1996). Surface roughness modulates the local pro- duction of growth factors and cytokines by osteoblast- like (MG63) cells. J Biomed Mater Res.; 32: 55-63

99. Lang NP, Berglundh T, Heitz-Mayfield LJ, Pjetursson BE, Salvi GE, Sanz M (2004) Consensus statements and recommended clinical procedures regarding implant survival and complications. Int J Oral Maxillofac Implants.;19:150-4.

100. Lee IK, Lim HC, Lee JS, Hong JY, Choi SH, Jung UW (2016) Layered approach with autogenous bone and bone substitute for ridge augmentation on implant dehiscence defects in dogs. Clin Oral Implants Res.; 27 (5): 622-8.

101. Lee J-S, Jung J-S, Im G-I, Kim B-S, Cho K-S, Kim C-S. (2015) Ridge regeneration of damaged extraction sockets using rhBMP-2: an experimental study in canine. J Clin Periodontol; 42: 678–687

102. LeGeros RZ, Lin S, Rohanizadeh R, Mijares D, LeGeros JP (2003) Biphasic calcium phosphate bioceramics: preparation, properties and applications. J Materials Science:Materials in Medicine; 14: 201-209

103. Li, H., Zheng, J., Zhang, S., Yang, C., Kwon, Y.D., Kim, Y.J. (2018) Experiment of GBR for repair of peri-implant alveolar defects in beagle dogs. Sci Rep. 8 (1): 16532

104. Lindhe J, Berglundh T, Ericsson I, Liljenberg B, Marinello C (1992) Experimental breakdown of peri-implant and periodontal tissues. A study in the beagle dog. Clin Oral Implants Res.;3(1):9-16.

105. Lundgren D, Laurell L, Gottlow J, Rylander H, Torbjörn M, Nyman S, Rask M (1995) The influence of the design of two different bioresorbable barriers on the results of guided tissue regeneration therapy. An intra-individual comparative study in the monkey. J Periodontol; 66: 605-612

106. Lundgren D, Sennerby L, Falk H, Friberg B, Nyman S (1994) The use of a new bioresorbable barrier for guided bone regeneration in connection with implant installation. Case reports. Clin Oral Implants Res 5: 177-184.

107. Lundgren, A. K., Lundgren, D., Sennerby, L., Taylor, A., Gottlow, J. & Nyman, S. (1997) Augmentation of skull bone using a bioresorbable barrier supported by autologous bone grafts. An intra-individual study in the rabbit. Clin Oral Implants Res 8, 90-95.

108. Lundgren, D., Mathisen, T., Gottlow, J. (1994) The development of a bioresorbable barrier for guided tissue regeneration. J Swed Dent Assoc. 86: 741-756

109. Lundgren, D., Slotte, C. (1999) Reconstruction of anatomically complicated periodontal de- fects using a bioresorbable GTR barrier supported by bone mineral. A 6-month fol- low-up study of 6 cases. J Clin Periodontol. 26: 56–62

110. Macthei EE (2001) The Effect of Membrane Exposure on the Outcome of Regenerative Procedures in Humans: A Meta-Analysis. J Periodontol; 72: 512-516

111. Maglione M, Vaccari L, Mancini L, Ciancio R, Bedolla DE, Bevilacqua L, Tonellato P (2019) Micro-ATR FTIR, SEM-EDS and X-ray micro-CT: an innovative multi-technique approach to investigate bone affected by peri-implantitis. Int J Oral Maxillofac Implants; 34 (3): 631–641

112. Mangano F, Gandolfi A, Luongo G, Logozzo S (2017). Intraoral scanners in dentistry: a review of the current literature. BMC Oral Health; 17: 149

113. Martins O, Ramos JC, Baptista IP, Dard MM (2014) The dog as a model for peri-implantitis. A review. J Investigative Surgery; 27: 50-56

114. Matsumoto G, Hoshino J, Kinoshita Y, Sugita Y, Kubo K, Maeda H, Ikada Y, Kinoshita Y (2012) Alveolar bone regeneration using poly-(lactic acid-co-glycolic acid-co-e-caprolactone) porous membrane with collagen sponge containing basic fibroblast growth factor: An experimental study in the dog. J Biomater Appl. 27 (4): 485-93

115. Mayfield, L., Soderholm, G., Halistrom, H., Kullendorff, B., Edwardsson, S., Bratthall, G., Brdgger, U., Attstrom, R. (1998) Guided tissue regeneration for the treatment of intraosseous defects using a bioabsorbable membrane. A controlled clinical study. J Clin Periodontol. 25: 585-595

116. Mehl A, Gloger W, Kunzelmann KH, Hickel R. A new optical 3-D device for the detection of wear. J Dent Res. 1997;76(11):1799-807.

117. Merli, M., Merli, I., Raffaelli, E., Pagliaro, U., Nastri, L., & Nieri, M. (2016). Bone augmentation at implant dehiscences and fenestrations. A sys- tematic review of randomised controlled trials. European Journal of Oral Implantology, 9, 11–32

118. Miller, N., Penaud, J., Foliguet, B., Membre, H., Amhrosini, P., Plombas, M. (1996) Resorption rates of 2 commercially available bioresorbable membranes. A hisiomorphometric study in a rabbit model. J Clin Periodontot. 23: 1051-1059

119. Mir-Mari, J., Benic, G. I., Valmaseda-Castellon, E., Hammerle, C. H. F. & Jung, R. E. (2017) Influence of wound closure on the volume stability of particulate and non-particulate GBR materials: an in vitro cone-beam computed tomographic examination. Part II. Clin Oral Implants Res 28 (6): 631-639

120. Moraschini V, da C. Poubel LA, Ferreira VF, dos SP. Barboza E (2015) Evaluation of survival and success rates of dental implants reported in longitudinal studies with a follow-up period of at least 10 years: a systematic review. Int. J. Oral Maxillofac. Surg.; 44: 377–388

121. Mordenfeld A, Hallman M, Johansson CB, Albrektsson T (2010) Histological and histomorphometrical analyses of biopsies harvested 11 years after maxillary sinus floor augmentation with deproteinized bovine and autogenous bone. Clin. Oral Impl. Res.; 21: 961–970

122. Moses O, Pitaru S, Artzi Z, Nemcovsky CE (2005) Healing of dehiscence-type defects in implants placed together with different barrier membranes: a comparative clinical study. Clin. Oral Impl. Res.;16: 210–219

123. Mouhyi J, Dohan Ehrenfest DM, Albrektsson T (2012) The per-implantitis: implant surfaces, microstructure, and physicochemical aspects. Clin Implant Dent Relat Res; 14 (2): 170-83

124. Naenni, N., Lim, H., Papageorgiou, S.N., Hammerle, C.H. (2019) Efficacy of lateral bone augmentation prior to implant placement: A systematic review and meta-analysis. J Clin Periodontol. 46 (Suppl. 21):287–306

125. Naenni, N., Schneider, D., Jung, R., Huesler, J., Hammerle, C., Thoma, D. (2017) Randomized clinical study assessing two membranes for guided bone regeneration of peri-implant bone defects: clinical and histological outcomes at 6 months. Clin. Oral Impl. Res. 28: 1309–1317

126. Nery EB, LeGeros RZ, Lynch KL, Lee K (1992) Tissue response to biphasic calcium phosphate ceramic with different ratio de HA/β-TCP in periodontal osseous defects. Periodontal; 63: 729-735

127. Nogueira LP, Braz D, Barroso RC, Oliveira LF, Pinheiro CJ, Dreossi D, Tromba G (2010) 3D histomorphometric quantification of trabecular bones by computed microtomography using synchrotron radiation. Micron.; 41(8): 990-6

128. Omar O, Elgali I, Dahlin C, Thomsen P (2019) Barrier membranes: More than the barrier effect? J Clin Periodontol. 46 (Suppl. 21):103–123.

129. Osborn J, Stoltenberg J, Huso B, Aeppli D, Pihlstrom B (1990) Comparison of measurement variability using a standard and constant force periodontal probe. J Periodontol 61: 497-503

130. Owens KW, Yukna RA (2001) Collagen membrane resorption in dogs: a comparative study. Implant Dent 10:49–56

131. Pearce AI, Richards RG, Milz S, Schneider E, Pearce SG (2007) Animal models for implants biomaterial research in bone: a review. European Cells and Materials;13: 1-10

132. Pjetursson BE, Thoma D, Jung R, Zwahlen M, Zembic A (2012) A systematic review of the survival and complication rates of implant-supported fixed dental prostheses (FDPs) after a mean observation period of at least 5 years. Clin. Oral Implants Res. 23(Suppl. 6), 22–38

133. Podaropoulos L, Veis AA, Papadimitriou S, Alexandridis C, Kalyvas D. Bone regeneration using beta-tricalcium phosphate in a calcium sulfate matrix. J Oral Implantol. 2009;35(1):28-36.

134. Polimeni G, Albandar JM, Wikesjö UM. Prognostic factors for alveolar regeneration: effect of space provision. J Clin Periodontol. 2005;32(9):951-4.

135. Qian W, Qiu J, Liu X (2019) Minocycline Hydrochloride Loaded Graphene Oxide Films On Implant Abutments For Peri-Implantitis Treatment In Beagle Dogs. J Periodontol., doi: 10.1002/JPER.19-0285

136. Quirynen M, Herrerra D, Teughels W, Sanz M (2014) Implant therapy: 40 years of experience. Periodontology 2000, 66; 7-12

137. Ramalingam, S., Al-Rasheed, A., ArRejaie, A., Noah, N., Al-Kindi, M., Al-Hezaimi, K. (2016) Guided bone regeneration in standardized calvarial defects using beta-tricalcium phosphate and collagen membrane: a real-time in vivo micro-computed tomographic experiment in rats. Odontology. 104 (2): 199-210

138. Ramanauskaite A, Becker K, Schwarz F (2018) Clinical characteristics of peri-implant mucositis and peri-implantitis. Clin Oral Impl Res.;29:551–556.

139. Rebele SF, Zuhr O, Schneider D, Jung RE, Hurzeler MB (2014) Tunnel technique with connective tissue graft versus coronally advanced flap with enamel matrix derivative for root coverage: a RCT using 3D digital measuring methods. Part II. Volumetric studies on healing dynamics and gingival dimensions. J Clin Periodontol.;41(6): 593-603.

140. Reinedahl D, Chrcanovic B, Albrektsson T, Tengvall P, Wennerberg A (2018) Ligature-Induced Experimental Peri-Implantitis-A Systematic Review. J. Clin. Med.; 7 (492): 1-9

141. Reinwald S & Burr D (2008) Review of nonprimate, large animal models for osteoporosis research. J Bone Miner Res.;23(9):1353-1368.

142. Renvert S, Persson GR, Pirih FQ, Camargo PM (2018) Peri-implant health, peri-implant mucositis, and peri-implantitis: Case definitions and diagnostic considerations. J Clin Periodontol.;45(Suppl 20): S278–S285.

143. Renvert S, Polyzois I, Claffey N (2011) How do implant surface characteristics influence peri- implant disease? J Clin Periodontol; 38 (Suppl. 11): 214–222.

144. Ricci A (2007) An objective method to measure soft tissue behavior around single-tooth implants. Part 1: Vertical measurements. European Journal of Esthetic Dentistry 2 (Suppl 4).

145. Roehling S, Gahlert M, Janner SFM, Meng B, Woelfler H, Cochran D (2019) Ligature-Induced Peri-implant Bone Loss Around Loaded Zirconia and Titanium implants. Int J Oral Maxillofacial Implants; 34 (2): 357-365

146. Rojo E, Stroppa G, Sanz-Martin I, Gonzalez-Martin O, Alemany AS, Nart J (2018) Soft tissue volume gain around dental implants using autogenous subepithelial connective tissue grafts harvested from the lateral palate or tuberosity area. A randomized controlled clinical study. J Clin Periodontol.;45: 495–503

147. Rothamel D, Schwarz F, Sager M, Herten M, Becker J (2005) Biodegradation of differently cross-linked collagen membranes: an experimental study in the rat. Clin. Oral Impl. Res; 16: 369–378

148. Rothamel D, Benner M, Finite T, Happe A, Kreppel M, Nickenig HJ, Zoller J (2014) Biodegradation pattern and tissue integration of native and cross-linked porcine collagen soft tissue augmentation matrices – an experimental study in the rat. Head & Face Medicine 10: 10

149. Rupp F, Liang L, Geis-Gerstorfer J, Scheideler L, Huttig F (2018) Surface characteristics of dental implants: A review. Dent Mater; 34 (1): 40-57

150. Sanz M & Vignoletti F (2015) Key aspects on the use of bone substitutes for bone regeneration of edentulous ridges. Dental Materials 31: 640-647

151. Sanz M, Dahlin C, Apatzidou D, et al. (2019) Biomaterials and regenerative technologies used in bone regeneration in the craniomaxillofacial region: Consensus report of group 2 of the 15th European Workshop on Periodontology on Bone Regeneration. J Clin Periodontol. 46 (Suppl. 21): 82–91.

152. Sanz M, Ferrantino L, Vignoletti F, de Sanctis M, Berglundh T (2017) Guided bone regeneration of non-contained mandibular buccal bone defects using deproteinized bovine bone mineral and a collagen membrane: an experimental in vivo investigation. Clinical Oral Implants Research 28: 1466-1476.

153. Sanz-Esporrin J, Blanco J, Sanz-Casado JV, Muñoz F, Sanz M (2019) The adjunctive effect of rhBMP-2 on the regeneration of peri-implant bone defects after experimental peri-implantitis. Clin Oral Impl Res; 30 (12): 1209-1219

154. Sanz Martin I, Vignoletti F, Nuñez J, Permuy M, Muñoz F, Sanz-Esporrin J, Fierravanti L, Shapira L, Sanz M (2017) Hard and soft tissue integration of immediate and delayed implants with a modified coronal macro design: Histological, micro CT and volumetric soft tissue changes from a pre-clinical in vivo study. Journal of Clinical Periodontology 44 (8):842-853

155. Sanz-Martin I, Benic GI, Hammerle CH, Thoma, DS (2016) Prospective randomized controlled clinical study comparing two dental implant types: volumetric soft tissue changes at 1 year of loading. Clin Oral Implants Res; 27: 406-411

156. Sanz-Martin I, Ferrantino L, Vignoletti F, Nunez J, Baldini N, Duvina M, Alcaraz J, Sanz M (2017) Contour changes after guided bone regeneration of large non-contained mandibular buccal bone defects using deproteinized bovine bone mineral and a porcine-derived collagen membrane: an experimental in vivo investigation. Clin Oral Investig 22(3): 1273-1283

157. Sanz-Martin I, Permuy M, Vignoletti F, Nuñez J, Muñoz F, Sanz M (2018) A novel methodological approach using superimposed Micro-CT and STL images to analyze hard and soft tissue volume in immediate and delayed implants with different cervical designs. Clin Oral Impl Res. 29: 986–995

158. Sanz-Martın I, Sailer I, Hammerle CHF, Thoma DS (2016). Soft tissue stability and volumetric changes after 5 years in pontic sites with or without soft tissue grafting: a retrospective cohort study.

159. Sanz-Sánchez I, Carrillo de Albornoz A, Figuero E, Schwarz F, Jung R, Sanz M, Thoma D (2018) Effects of lateral bone augmentation procedures on peri-implant health or disease: A systematic review and meta-analysis. Clin Oral Impl Res. 29 (Suppl. 15):18–31

160. Sanz-Sanchez I, Ortiz-Vigon A, Sanz-Martin I, Figuero E, Sanz M (2015) Effectiveness of Lateral Bone Augmentation on the Alveolar Crest Dimension: A Systematic Review and Meta-analysis. J Dent Res.; 94 (9 Suppl):128S-42S.

161. Sason GK, Mistry G, Tabassum R, Shetty O (2018) A comparative evaluation of intraoral and extraoral digital impressions: An in vivo study. J Indian Prosthodont Soc; 18 (2): 108-116

162. Saulaci N & Schaller B (2019) Prevalence of Peri-Implantitis in Implants with Turned and Rough Surfaces: a Systematic Review. J Oral Maxillofac Res; 10(e1): 1-12

163. Schneider D, Ender A, Truninger T, Leutert C, Sahrmann P, Roos M, Schmidlin P (2014) Comparison between clinical and digital soft tissue measurements. J Esthet Restor Dent 26: 191-199

164. Schneider D, Grunder U, Ender A, Hammerle CH, Jung RE. Volume gain and stability of peri-implant tissue following bone and soft tissue augmentation: 1-year results from a prospective cohort study. Clin Oral Implants Res. 2011;22(1):28-37.

165. Schneider D, Weber FE, Grunder U, Andreoni C, Burkhardt R, Jung RE (2014). A randomized controlled clinical multicenter trial comparing the clinical and histological performance of a new, modified polyglycolide acid membrane to an expanded polytetrafluorethylene membrane in guided bone regeneration procedures. Clin. Oral Impl. Res.; 25: 150–158

166. Schou S, Holmstrup P, Stoltze K, Hjørting-Hansen E, Fiehn NE, Skovgaard LT (2002). Probing around implants and teeth with healthy or inflamed peri-implant mucosa/gingiva. Clin. Oral Impl. Res;13: 113–126

167. Schropp, L., Wenzel, A., Kostopoulos, L. & Karring, T. (2003) Bone healing and soft tissue contour changes following single-tooth extraction: a clinical and radiographic 12-month prospective study. Int J Periodontics Restorative Dent 23, 313-323.

168. Schwarz F, Derks J, Monje A, Wang HL (2018) Peri-implantitis. J Clin Periodontol.;45(Suppl 20):S246–S266.

169. Schwarz F, Herten M, Ferrari D, Wieland M, Schmitz L, Engelhardt E, et al (2007) Guided bone regeneration at dehiscence-type defects using biphasic hydroxyapatite + beta tricalcium phosphate (Bone Ceramic) or a collagen-coated natural bone mineral (BioOss Collagen): an immunohistochemical study in dogs. Int J Oral Maxillofac Surg.; 36 (12): 1198-206.

170. Schwarz F, Herten M, Sager M, Bieling K, Sculean A, Becker J (2007) Comparison of naturally occurring and ligature- induced peri-implantitis bone defects in humans and dogs. Clin. Oral Impl. Res., 18; 161–170

171. Schwarz F, Rothamel D, Herten M, Sager M, Becker J (2006) Angiogenesis pattern of native and cross-linked collagen membranes: an immunohistochemical study in the rat. Clin. Oral Impl. Res; 17: 403–409

172. Schwarz F, Rothamel D, Herten M, Sager M, Ferrari D, Becker J (2008) Immunohistochemical characterization of guided bone regeneration at dehiscence-type defect using different barrier membranes: an experimental study in dogs. Clin. Oral Impl. Res; 19: 402–415

173. Sculean A, Gruber R, Bosshardt DD (2014) Soft Tissue Wound Healing Around Teeth and Dental Implants. J Periodontol.;4:6–22.

174. Simion M, Baldoni M, Rossi P, Zaffe D (1994) A comparative study of the effectiveness of e-PTFE Membranes with and without early exposure during the healing period. Int J Periodont Rest Dent; 14: 167-180

175. Song JM, Shin SH, Kim YD, Lee JY, Baek YJ, Yoon SY, Kim HS (2014) Comparative study of chitosan/fibroin–hydroxyapatite and collagen membranes for guided bone regeneration in rat calvarial defects: micro-computed tomography analysis. Int J Oral Sci. 6 (2): 87-93

176. Strebel J, Ender A, Paque F, Krahenmann M, Attin T, Schmidlin PR. In vivo validation of a three-dimensional optical method to document volumetric soft tissue changes of the interdental papilla. J Periodontol. 2009;80(1):56-61.

177. Tan WL, Wong TLT, Wong MCM, Lang NP (2012) A systematic review of post-extractional alveolar hard and soft tissue dimensional changes in humans. Clin. Oral. Impl. Res.; 23(Suppl. 5): 1–21

178. Tanuma, Y., Matsui, K., Kawai, T., Matsui, A., Suzuki, O. & Kamakura, S. (2013) Comparison of bone regeneration between octacalcium phosphate/collagen composite and beta-tricalcium phosphate in canine calvarial defect. Oral Surgery Oral Medicine Oral Pathology Oral Radiology 115 (Suppl 1), 9-17.

179. Thoma DS, Bienz SP, Figuero E, Jung RE, Sanz-Martín I (2019) Efficacy of lateral bone augmentation performed simultaneously with dental implant placement: A systematic review and meta-analysis. J Clin Periodontol. 46 (Suppl. 21): 257–276.

180. Thoma DS, Jung RE, Schneider D, Cochran DL, Ender A, Jones AA, et al. Soft tissue volume augmentation by the use of collagen-based matrices: a volumetric analysis. J Clin Periodontol. 2010;37(7):659-66.

181. Thoma DS, Jung U-W, Park J-Y, Bienz SP, Hursler J, Jung RE (2017). Bone augmentation at peri-implant dehiscence defects comparing a synthetic polyethylene glycol hydrogel matrix versus standard guided bone regeneration techniques. Clin. Oral Impl. Res. 28, e76–e83.

182. Trisi, P., Rao, W., Rebaudi, A. & Fiore, P. (2003) Histologic effect of pure-phase beta-tricalcium phosphate on bone regeneration in human artificial jawbone defects. International Journal of Periodontics & Restorative Dentistry 23 (Suppl 1), 69-77.

183. Tromhelli, L., Scabbia, A., Talakis, D.N., Checchi, L., Calura, G. (1998) Resorbable barrier and envelope flap surgery In ihe treatment of human gingival recession defects. Case Reports. J Clin Periodontol: 25: 24-29.

184. Trombelli L, Farina R, Marzola A, Bozzi L, Liljenberg B, Lindhe J (2008). Modeling and remodeling of human extraction sockets. J Clin Periodontol; 35: 630–639

185. Van Assche N, Michels S, Naert I, Quirynen M. Randomized controlled trial to compare two bone substitutes in the treatment of bony dehiscences. Clin Implant Dent Relat Res. 2013;15(4):558-68.

186. Van der Weijden F, Dell'Acqua F, Slot DE. Alveolar bone dimensional changes of post-extraction sockets in humans: a systematic review. J Clin Periodontol. 2009;36(12):1048-58.

187. Varon-Shahar E, Shusterman A, Piattelli A, Iezzi G, Weiss EI, Houri-Haddad Y (2019) Peri-implant alveolar bone resorption in an innovative peri-implantitis murine model: Effect of implant surface and onset of infection. Clin Implant Dent Relat Res.; 21 (4): 723-733.

188. Viornery C, Chevolot Y, Léonard D, Aronsson BO, Péchy P, Mathieu HJ, Descouts P, Grätzel M (2002). Surface modification of titanium with phosphonic acid to improve bone bonding: Characterization by XPS and ToF-SIMS. Langmuir; 18, 2582–2589.

189. Viornery C, Guenther HL, Aronsson BO, Péchy P, Descouts P, Grätzel M (2002). Osteoblast culture on polished titanium disks modified with phosphonic acids. J. Biomed. Mater. Res.; 62, 149–155.

190. Von Arx T, Cochran DL, Hermann JS, Schenk RK, Higginbottom FL, Buser D. Lateral ridge augmentation and implant placement: an experimental study evaluating implant osseointegration in different augmentation materials in the canine mandible. Int J Oral Maxillofac Implants. 2001;16(3):343-54

191. Von Salis-Soglio M, Stübinger S, Sidler M, Klein K, Ferguson S, Kämpf K, et al. (2014). A Novel Multi-Phosphonate Surface Treatment of Titanium Dental Implants: A Study in Sheep. J. Funct. Biomater; 5 (3): 135–57

192. Wessing B, Urban I, Montero E, Zechner W, Hof M, Al andez Chamorro J, Alandez Martin N, Polizzi G, Meloni S, Sanz M. (2017) A multicenter randomized controlled clinical trial using a new resorbable non-cross-linked collagen membrane for guided bone regeneration at dehisced single implant sites: interim results of a bone augmentation procedure. Clin. Oral Impl. Res. 28, e218–e226

193. Windisch SI, Jung, RE, Sailer I, Studer SP, Ender A, Hämmerle CHF (2007) A new optical method to evaluate three-dimensional volume changes of alveolar contours: a methodological in vitro study. Clinical Oral Implants Research 18: 545-551

194. Wiranto MG, Engelbrecht WP, Tutein Nolthenius HE, van der Meer WJ, Ren Y (2013) Validity, reliability, and reproducibility of linear measurements on digital models obtained from intraoral and cone-beam computed tomography scans of alginate impressions. Am J Orthod Dentofacial Orthop.;143 (1) :140-7.

195. Won JY, Park CY, Bae JH, Ahn G, Kim C, Lim DH, Cho DW, Yun WS, Shim JH (2016) Evaluation of 3D printed PCL/PLGA/-TCP versus collagen membranes for guided bone regeneration in a beagle implant model. Biomed Mater. 11 (5): 055013

196. Yamada S, Heymann D, Bouler JM, Daculsi G (1997) Osteoclastic resort3tion of calcium phosphate ceran&s with different hydroxyapatite/β-tricalcium phosphate ratios. Biomaterials; 18:1037-1041

197. Zeltner M, Jung RE, Hammerle CHF, Hu€sler J, Thoma DS. Randomized controlled clinical study comparing a volume-stable collagen matrix to autogenous connective tissue grafts for soft tissue augmentation at implant sites: linear volumetric soft tissue changes up to 3 months. J Clin Periodontol 2017; 44: 446–453

198. Zhao S, Pinholt EM, Madsen JE, Donath K (2000) Histological evaluation of different biode- gradable and nonbiodegradable mem- branes im- planted sub-cutaneously in rats. J Craniomaxillofac Surg 28:116–122

199. Zitzmann NU, Naef R, Scharer P (1997) Resorbable Versus Nonresorbable Membranes in Combination With Bio-Oss for Guided Bone Regeneration. Int J Oral Maxillofacial Implants; 12 (6): 844-852

200. Zitzmann, N.U., Schärer, P., Marinello, C.P. (2001) Long-term results of implants treated with guided bone regeneration: a 5-year prospective study. International Journal of Oral and Maxillofacial Implants 16(3):355-66.

XI. ANEXOS

Imágenes Suplementarias

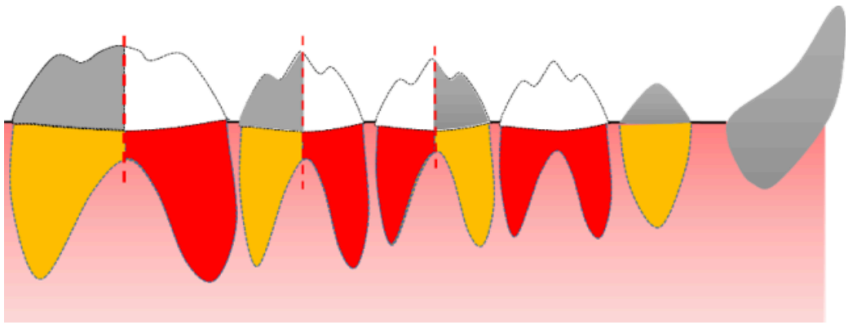


Imagen Suplementaria 1. Representación gráfica de las extracciones realizadas en las mandíbulas de los perros en los estudios de regeneración ósea (estudios 1 y 2)

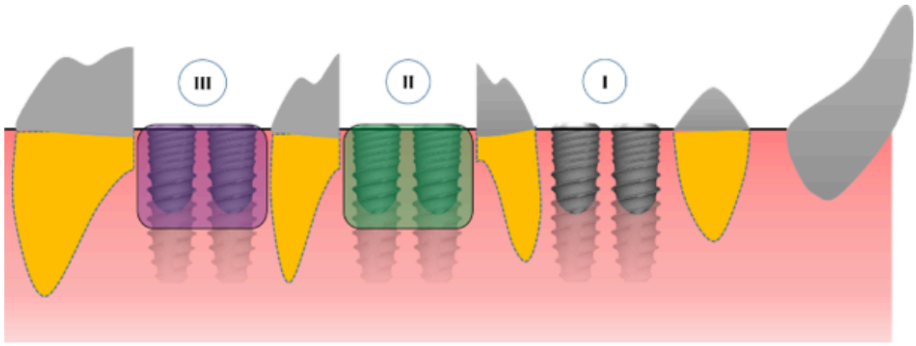


Imagen Suplementaria 2. Representación gráfica de la asignación de los tres grupos en el estudio 1 de regeneración ósea (I: control negativo, II control positivo, III test)

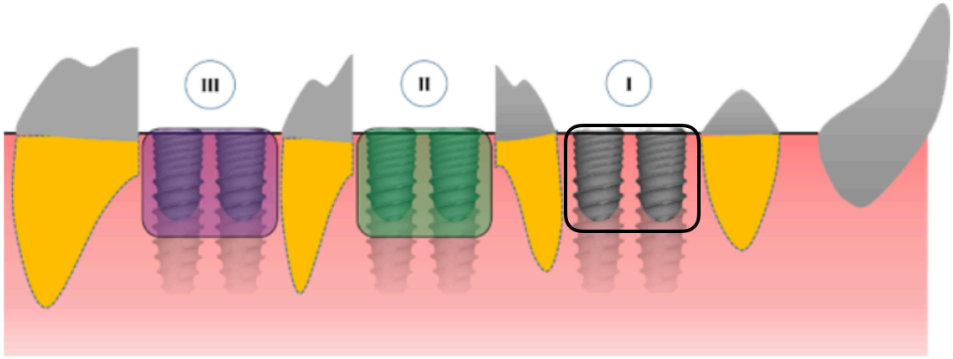


Imagen Suplementaria 3. Representación gráfica de la asignación de los tres grupos en el estudio 2 de regeneración ósea (I: control negativo, II control positivo, III test)

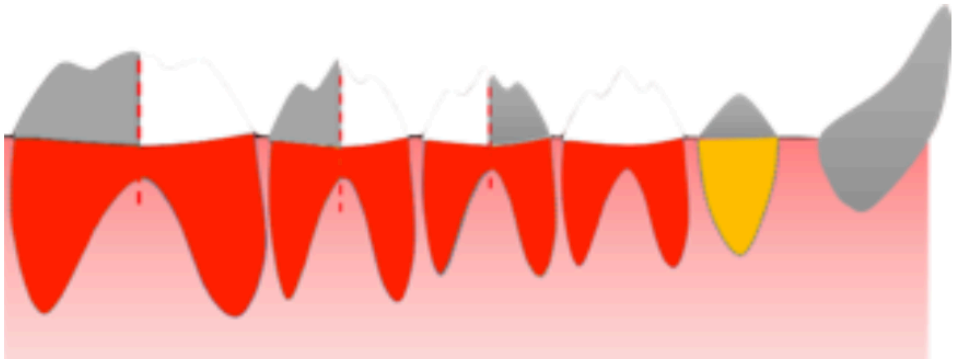


Imagen Suplementaria 4. Representación gráfica de las extracciones realizadas en las mandíbulas de los perros en el estudio de peri-implantitis